Multistep Modelling and Monitoring of Bioprocesses

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Abstract: *A new approach is proposed for modelling and monitoring bioprocesses dynamics characterized by different metabolic states. Bioprocesses cannot be described by a single model. For this reason, three phases characterised by the bioprocess are defined – periodic, exponential, and stationary. During each phase, the process passes through one or more physiological states. Each physiological state is described by a sub-model with a different structure and parameter values. The transition of the process from one physiological state to another is carried out by switching the sub-models based on a predefined key parameter. Monitoring is performed by a cascade of software sensors using the sub-models and real-time measurement of the concentrations of the main process variables. The proposed approach was tested by modelling and monitoring the Escherichia coli phytase production process.*

Keywords: *Modelling, Monitoring, Bioprocesses, Escherichia coli.*

Introduction

Accurate monitoring of all conditions inherent in individual microorganism cultivation processes is necessary for the optimization of bioprocess production. Unfortunately, access to sensors that meet the requirements for online process monitoring is limited. One solution to this problem is to combine hardware sensors with algorithms for online estimation of unmeasured variables and parameters, known as software sensors (SS) [4]. Currently, SS have established themselves as promising tools for monitoring various bioprocesses [1, 3, 4, 6-8, 11-13, 14, 20-22, 27]. In [12, 28], reviews of SS based on models and data have been made. The selection of an SS method for a specific biotechnological process requires analysis of the following information: (i) complexity of the specific process; (ii) degree of knowledge about system dynamics; (iii) quality and quantity of available measurements, types of noise and uncertainties, etc. Much of the research in the field of model-based SS is based on the development of complex nonlinear extended Kalman filter algorithms, in which there is no guarantee of convergence and stability [7, 12]. Other SS monitoring approaches are based on adaptive system theory [1, 9, 10, 15, 16, 18, 23], high gain approach [2, 25, 26], sliding mode theory [5, 14], SS interval [17], probabilistic observers [3], etc. All these methods are highly dependent, to varying degrees, on knowledge of the kinetics of the process. Regarding the

monitoring of processes that pass through different physiological states (metabolic regimes) and are characterized by multiple specific growth rates, only a few studies [19, 24, 29] concerning the application of SS have been proposed in the literature. Such processes, for example, include intermediate metabolites (acetate at high-cell density fermentation of *Escherichia coli* and phytase production by *E. coli*, ethanol at baker yeast production by *Saccharomyces cerevisiae*, gluconic acid at fermentation of *Aspergillus niger*, etc.) that are produced and could be consumed during the cultivation.

The considered class of processes is described by the following reaction scheme, including three reaction rates:

oxidative growth on the substrate

$$k_1 S \xrightarrow{\mu_1} X + k_5 P, \tag{1}$$

fermentative growth on the substrate

$$k_2 S \xrightarrow{\mu_2} X + k_3 M + k_6 P, \tag{2}$$

oxidative growth on the intermediate metabolite

$$k_4 M \xrightarrow{\mu_3} X + k_7 P, \tag{3}$$

where X, S, M and P are concentrations of biomass, main substrate, intermediate metabolite, and target product, respectively; $k_1 \div k_7$ are yield coefficients; $\mu_1 \div \mu_3$ are specific growth rates, related to the different reaction rates.

Based on the reaction scheme (1)-(3), physiological states could be described by combinations of sub-models, presenting the process dynamics. One challenge is finding reliable information for switching from one metabolic state to another and switching the sub-models.

In [23], the oxidative capacity presented by a model with constant parameters is proposed as a key parameter (marker) for switching the sub-models. As a result, inaccuracies in the estimation are observed, since the values are in a close relationship with the type of the strain and the cultivation conditions. A new marker for recognizing the regimen bottlenecks is the kinetics of intermediate metabolites, proposed in [29, 30]. The process was monitored by a cascade scheme of the SS, which changes its structure depending on the sign of the marker. At the scheme input are the real-time measurements of the main substrate and the intermediate metabolite, and at the output are the immeasurable variables and parameters.

All the proposed solutions mentioned above are based on sub-models of the considered process, whose parameters do not change during the process. When the complexity of the process is higher and changes in the state of the process occur very quickly within one phase – periodic, exponential, and stationary, the proposed sub-models with constant coefficients cannot give accurate results due to the non-linear and non-stationary nature of the considered processes.

This paper proposes a new method for modelling and online monitoring of bioprocesses that cannot be described by a single model. Three phases, periodic, exponential and stationary, characterizing the bioprocess are defined. During each of the phases, the process passes through one or more physiological states. Each physiological state is described by a sub-model with

a different structure. The sub-model switching is based on a predefined adaptive key parameter. Based on a cascade of SS using the sub-models and real-time measurement of the concentrations of the main substrate and intermediate metabolite, monitoring is carried on. The proposed approach is tested by modelling and monitoring the phytase production process by *E. coli* BL21(DE3)pPhyt109.

For the realization of the new method, a four-step cascade scheme of SS is developed. Inputs of the scheme are online measurements of glucose (main substrate) and acetate (intermediate metabolite) concentrations. In the first step, the SS of acetate production or consumption rates are designed using acetate measurements. The outputs of the scheme are the specific biomass growth rates and the biomass and target product (phytase) concentrations. Conclusions about the applicability of the proposed approach are made.

Materials and methods

Operational models of a class bioprocesses

The class processes go through three metabolic regimes according to the scheme (1)-(3). A key parameter (marker) that adaptively recognizes the change of metabolic regimes of the process and on this basis switches the various sub-models that describe these regimes, is applied. The key parameter is the kinetics of the intermediate metabolite (production or consumption), information on which can be obtained from real-time measurements of this metabolite.

The model (4) describing the oxidative-fermentative growth of biomass on glucose (4.1) and oxidative growth on intermediate metabolite (*M*) is shown in Fig. 1. It is represented by two sub-models, where *F* is substrate feed rate, *V* is the reactor volume and S_{in} is the concentration of feeding substrate. When the marker accepts positive or zero values, the sub-model describing the oxidative-fermentative growth on the main substrate is included. Negative values of the marker are an indicator of metabolite consumption and transition to oxidative growth on the main substrate and metabolite.

oxidative-fermentative growth on substrate

$$\phi_M \geq 0 \quad \Rightarrow \quad \frac{d}{dt}\begin{bmatrix} X \\ S \\ M \\ P \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ -k_1 & -k_1 \\ 0 & k_3 \\ k_5 & k_6 \end{bmatrix} \begin{bmatrix} \mu_1 \\ \mu_2 \end{bmatrix} X - \frac{F}{V} \begin{bmatrix} X \\ S - S_{in} \\ M \\ P \end{bmatrix} \tag{4.1}$$

Marker

$$\phi_M = \frac{dM}{dt} + \frac{F}{V} M$$

oxidative growth on intermediate metabolite

$$\phi_M < 0 \quad \Rightarrow \quad \frac{d}{dt}\begin{bmatrix} X \\ S \\ M \\ P \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ -k_1 & 0 \\ 0 & -k_4 \\ k_5 & k_7 \end{bmatrix} \begin{bmatrix} \mu_1 \\ \mu_3 \end{bmatrix} X - \frac{F}{V} \begin{bmatrix} X \\ S - S_{in} \\ M \\ P \end{bmatrix} \tag{4.2}$$

Fig. 1 Two sub-models describing the dynamics of the class of controlled processes

On-line estimation of the rate of metabolite production

According to the general approach to estimating all kinetics as an unknown non-stationary parameter [10, 12], the SS for the rate of metabolite production has the following form:

$$\frac{d\hat{M}}{dt} = \hat{\Phi}_{мр} - \frac{F}{V}M + w_1(M - \hat{M}), \quad (5.1)$$

$$\frac{d\hat{\Phi}_{мр}}{dt} = w_2(M - \hat{M}), \quad (5.2)$$

where $\hat{\Phi}_{мр}$ and $\hat{M}$ are the estimates of metabolite production rate and metabolite concentration respectively; and M is the measured values of metabolite; and w_1 and w_2 are tuning parameters of the SS (5).

Software sensors of biomass growth rate on the substrate are presented as:

$$\frac{d\hat{S}}{dt} = -k_1\hat{R}_{X1} - k_2\hat{R}_{X2} + \frac{F}{V}(S_{in} - S) + w_3(S - \hat{S}), \quad (6.1)$$

$$\frac{d\hat{R}_{X1}}{dt} = w_4(S - \hat{S}), \quad (6.2)$$

where $\hat{R}_{X1}$ and $\hat{R}_{X2}$ are estimates of biomass growth rates in oxidation and fermentation on the substrate, respectively, and w_3 и w_4 are tuning parameters of the SS (6).

The estimates of $\hat{R}_{X2}$ are obtained using the following relation:

$$\hat{R}_{X2} = \hat{\Phi}_{мр}/k_3. \quad (7)$$

The tuning of the SS will be demonstrated by a case study.

On-line estimation of the rate of metabolite consumption

The structure of this SS is similar to (5) as follows:

$$\frac{d\hat{M}}{dt} = \hat{\Phi}_{мс} - \frac{F}{V}M + w_5(M - \hat{M}), \quad (8.1)$$

$$\frac{d\hat{\Phi}_{мс}}{dt} = w_6(M - \hat{M}), \quad (8.2)$$

where $\hat{\Phi}_{мс}$ is the estimate of metabolite consumption rate, and w_5 и w_6 are tuning parameters of the SS (8).

The tuning of the SS (5), (6), and (8) will be demonstrated by a case study. Using the estimates of metabolite consumption rate ($\hat{\Phi}_{мс}$) the estimation of oxidative growth rate on metabolite, biomass, target product, and specific growth rates could be obtained as presented bellow.

Online estimation of oxidative growth rate on metabolite, biomass, target product, and specific growth rates

The estimates of biomass growth rate in oxidation on metabolite, $\hat{R}_{X3}$, are obtained using the relationship as follows:

$$\hat{R}_{X3} = -\hat{\Phi}_{мр}/k_4. \quad (9)$$

Based on SS (5)-(9) the estimates of biomass ($\hat{X}$) and target product ($\hat{P}$) can be calculated using the equations:

$$\frac{d\hat{X}}{dt} = \hat{R}_{X1} + \hat{R}_{X2} + \hat{R}_{X3} - \frac{F}{V}\hat{X}, \quad (10)$$

$$\frac{d\hat{P}}{dt} = k_5\hat{R}_{X1} + k_6\hat{R}_{X2} + k_7\hat{R}_{X3} - \frac{F}{V}\hat{P}. \quad (11)$$

These results allow the values of specific growth rates μ_1, μ_2 and μ_3 to be received:

$$\hat{\mu}_1 = \hat{R}_{X1}/\hat{X}, \quad (12.1)$$

$$\hat{\mu}_2 = \hat{R}_{X2}/\hat{X}, \quad (12.2)$$

$$\hat{\mu}_3 = \hat{R}_{X3}/\hat{X}. \quad (12.3)$$

The relationships between SS for the monitoring of the considered class of processes are given in Fig. 2.

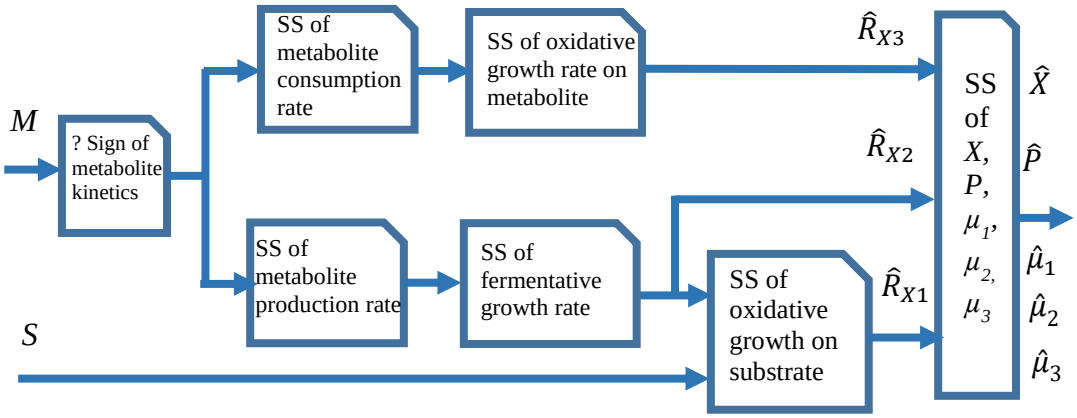

Fig. 2 Cascade scheme of software sensors for process monitoring

Results and discussion

Case study – modelling and monitoring of fed-batch process for phytase production

The experimental data for extracellular production of bacterial phytase fed-batch cultivation of *E. coli* strain BL21(DE3)pPhyt109 are carried out in the Department of Fermentation Engineering, Faculty of Technology, University of Bielefeld [24]. Analysing the experimental data (Fig. 3, black stars), three phases are clearly outlined – periodic, exponential and stationary, as for each of them, one or two metabolic regimes appear. For the considered case, the main substrate is glucose, the intermediate metabolite is acetate and the target product is phytase. As can be seen from the acetate experimental data, in each phase production and consumption of acetate is observed. For this reason, each phase has to be described with the proposed model from Fig. 1. This requires the identification of the group of sub-models at each phase.

For the purposes of the simulation investigations, the following kinetic expressions are used:

$$\mu_1 = q_{s,crit}/k_1, \tag{13.1}$$

$$\mu_2 = (q_s - q_{s,crit})/k_2, \tag{13.2}$$

$$\mu_3 = q_{ac}/k_4, \tag{13.3}$$

where $q_{s,crit} = \frac{q_{o,max}}{k_{os}} \frac{K_{i,o}}{K_{i,o}+A}$ and $q_{ac} = q_{ac,max} \frac{A}{A+K_A} \frac{K_{i,A}}{K_{i,A}+A}$.

In the system (13) $q_{s,crit}$, $q_{o,max}$, k_{os}, $K_{i,o}, q_{ac,max}$, $K_{i,A}$, K_A are kinetic constants.

The identification of the models (4) with the expressions (13) under the criterion of minimum root mean square error between the model and experimental data is performed. The results are given in Table 1.

Table 1. Optimal values of model parameters

Phase	q_{smax}	k_s	k_{is}	q_{omax}	k_{os}	k_{io}	q_{amax}	k_a	k_{ia}	k_1	k_2	k_3	k_4	k_5	k_6	k_7
1	4.2	0.19	5.54	1.1	2.15	0.1	0.08	1.17	-	3.69	0.56	0.19	4.6	1.4	2.7	0.45
2	34.2	0.79	1.83	0.5	2.5	0.2	0.143	0.97	0.25	2.08	2.17	0.05	4.1	2.9	1.5	0.5
3	77.1	0.47	12.3	2.1	3.3	0.13	0.002	0.3	0.23	16.6	11.7	0.42	9.9	39.4	9.5	0.56

The values of the kinetic parameters of the models for the three phases are compared in the table. In general, they have significant differences in values with few exceptions. This is an expected fact from the point of view of the non-stationary nature of the bioprocesses and the different dynamics during the different phases.

The proposed new method for modelling bioprocesses makes it possible to describe with good accuracy the dynamics of complex processes that pass through different physiological states and different growth phases, as well as are characterized by several specific growth rates. The obtained results are shown in Fig. 3, where, for each phase, a comparison between experimental and model data is given. In all figures, with black lines and points are the results of the modelling and monitoring of the periodic phase, with red lines and points – of the exponential and with blue lines and points – of the stationary phase.

The monitoring of this process is realized by applying the SS (5)-(12) based on the cascade scheme shown in Fig. 2. The tuning of SS is carried out assuming that there is equality between the eigenvalues of the system of estimation errors as $h_1 = h_2 = h$ with h – a negative constant to ensure the stability of SS (5)-(12). In this way, the tuning procedure is reduced to the choice of one parameter (h) for each SS. The design parameters are calculated as follows: $w_i = -2h$, $w_j = w_i^2/4$, where i = 1, 3, 5, j = 2, 4, 6, and h = -10.

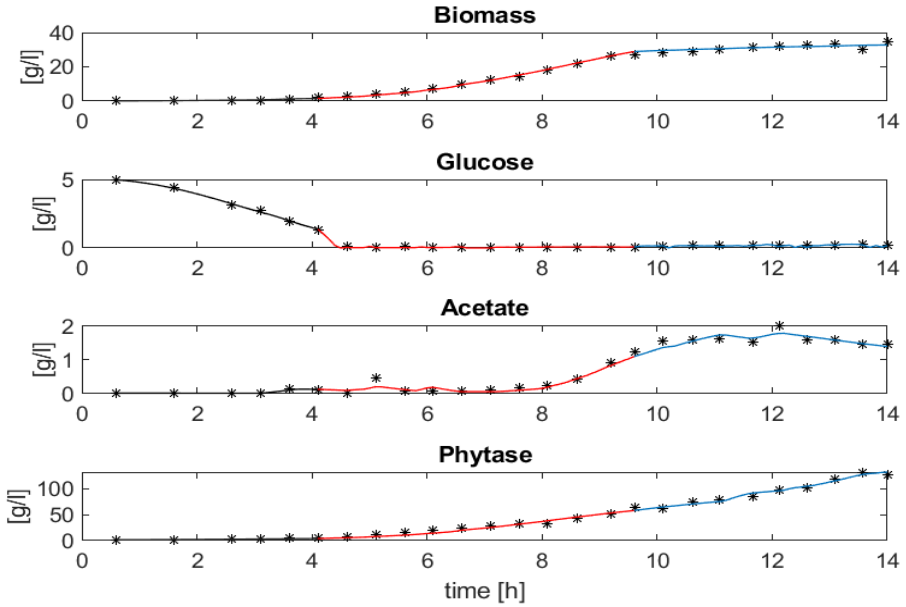

Fig. 3 Comparison between experimental and model data for the main process variables

Fig. 4 shows the estimation results of acetate production and consumption rates. The dashed lines delineate parts of the different phases of the process, where it is clearly seen that the production and consumption of this metabolite alternate in each phase. As can be seen from the results, the estimation quality is good when compared to the model.

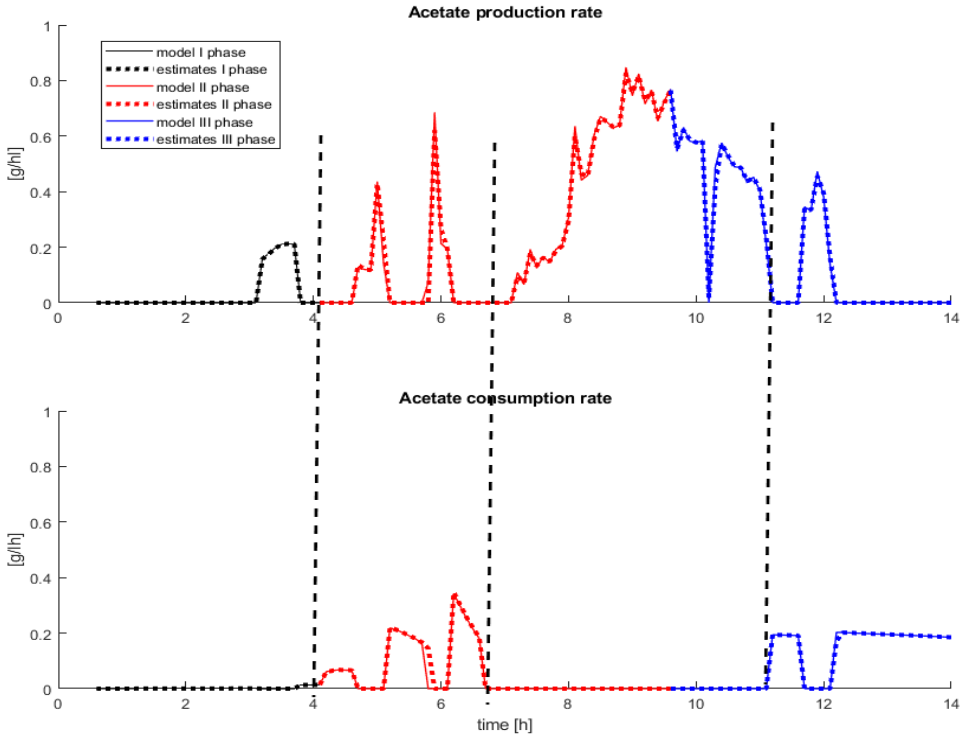

Fig. 4 Model (lines) and estimated values (points) of the acetate production and consumption rates

The results for the specific growth rates were obtained based on Eqs. (12), are shown in Fig. 5. The comparison of the estimates with the model data for all three specific rates shows a good tracking of their dynamics.

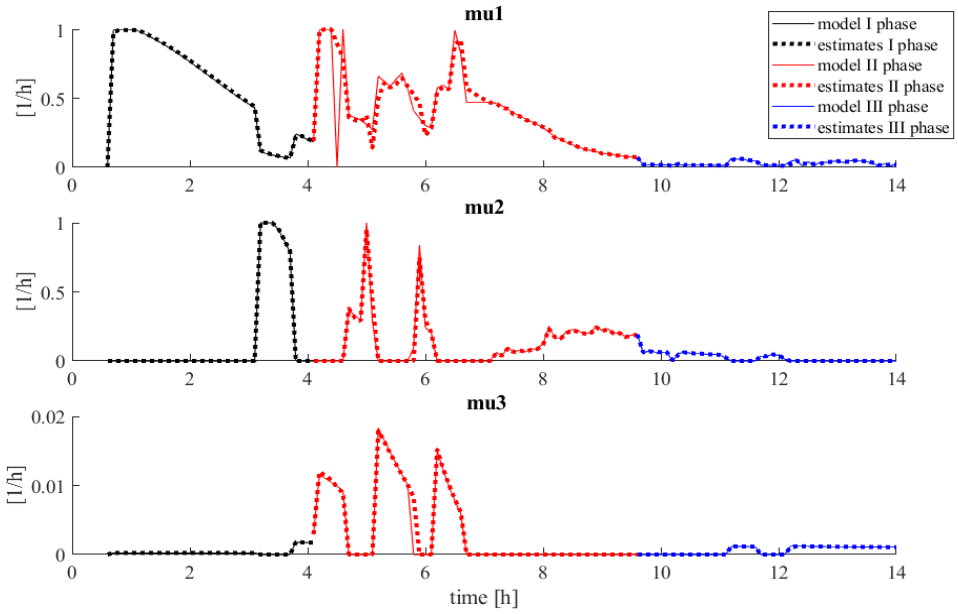

Fig. 5 Model (lines) and estimated values (points) of the three specific growth rates

Fig. 6 shows the results of the estimation of the biomass and the target product – phytase obtained from Eqs. (10) and (11). The estimates were compared to the experimental data for each of the phases.

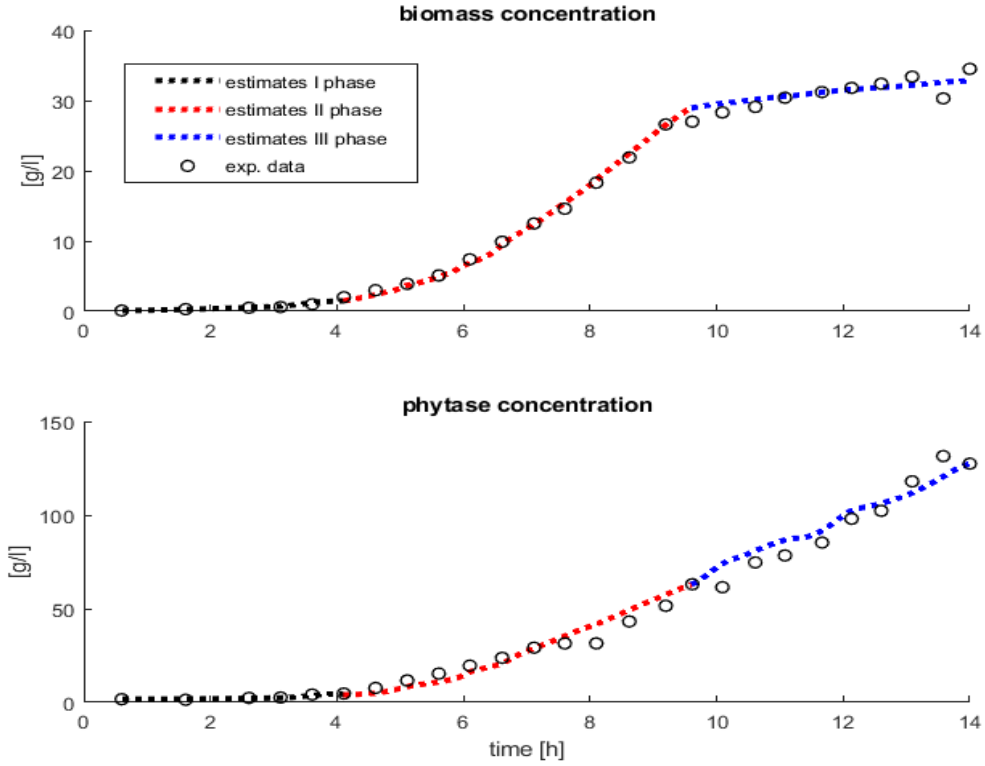

Fig. 6 Experimental (circles) and estimated values (points) of concentrations of biomass and target product

Discussion

The proposed approach for modelling and monitoring bioprocesses with different metabolic states allows a good description of the process dynamics of the fed-batch cultivation of *E. coli* BL21(DE3)pPhyt109 for the extracellular production of bacterial phytase. This is due both to dividing the process into three phases and to the proposed sub-models, switching from a key parameter, which describes the change of physiological states for each phase of the process. An evolutionary algorithm was applied for the parametric identification of the model. It is realized using optimization under criteria minimal mean square errors between experimental data of the main process variables and corresponding model data. The three phases were modelled with good accuracy as can be seen in Fig. 3. Based on the comparison of the experimental data of biomass and phytase and estimated ones (Fig. 6), it can be observed that the results for the first and the second phase are more accurate in comparison to the third phase. This is due to changes in the dynamics of the intermediate metabolite, which determines the frequent switching of sub-models and SS. The process kinetics, represented by the dynamics of the specific growth rates (Fig. 5), corresponds to the physiological states through which the process passes. As can be seen from the results presented in Fig. 4, the production and consumption of the intermediate metabolite acetate is consistent with the experimental data and is reflected in the dynamics of the specific rates μ_2 and μ_3.

Conclusion

The proposed new method for modelling and monitoring bioprocesses makes it possible to describe with good accuracy the dynamics of complex processes that pass through different physiological states and different growth phases, as well as are characterized by several specific growth rates.

The proposed approach along with other monitoring and control models and algorithms will be embedded in the software package Bioprocess Interactive Modeling and Control System (InSEMCoBio) to train bioengineering students and professionals with modern modelling methods (http://insemcobio.ir.bas.bg/).

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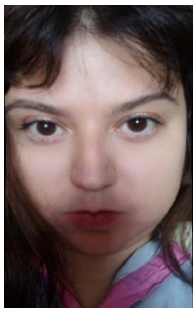

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