DE GRUYTER OPEN

Print ISSN: 1312-2622; Online ISSN: 2367-5357
DOI: 10.1515/itc-2016-0016

Monitoring the Kinetics of Bioprocess Variables – Theory and Applications

V. Lyubenova

Key Words: *Model-based software sensors; biotechnological processes; kinetics estimation.*

Abstract. *A new method for monitoring of bioprocess kinetics is developed where there exists a set of measured variables but the use of constant values of kinetic parameters is not acceptable. The method is based on new formalization of kinetics of biotechnological processes. It is generalized in one vector including unknown time-varying parameters only. For kinetics estimation, a general structure of software sensor is presented. A optimization procedure for tuning of the sensor's parameters is proposed on the basis of stability and convergence analysis. The proposed method is applied for monitoring of bioprocesses for gluconic acid, alpha-amylase and ethanol production.*

I. Introduction

The advancement of bioprocess monitoring will play a crucial role to meet the future requirements of bioprocess technology. The software sensors gain popularity as basic tool for the monitoring of main process variables and parameters. They exist a lot of methods for software sensors design in the literature, whose structure depends on aprior process information and statistical significance of the experimental data. The lack of exact statistical characteristics leads to application of deterministic methods mainly model based ones. The General Dynamical Model of Bioreactors [1] is a contemporary global and rigorous theoretical framework for analysis of bioreactor dynamics. It proposes software sensors designed as observer-based estimators. The structure of considered general estimator is derived by acception that the process kinetics is partially known and formalized as production of matrix of known constant yield coefficients and vector of process reaction rates considered as unknown time-varying parameters. A main advantage is its linear structure allowing to overcome the problems resulting from the nonlinear dynamics of these processes. Different tuning procedures of considered estimator' parameters are applied in [2-4]. In [4], a systematic procedure that allows a 'user's friendly' tuning is proposed. This approach had a lot of successful applications in the cases when the yield coefficients could be accepted as constants [5-9]. The bioprocesses carried out in industrial instalations are forced to work in uncertainty conditions – inhomogeneous and/or multicomponent cultivation medium. The use of constant kinetic parameters is not acceptable for considered processes. The lack of information for their true values is the reason the process kinetics to be considered as fully unknow. In this paper, a method for estimation of unknown process kinetics is proposed. It is developed by new formalization of kinetics of measurable variables considered as unknown time-varying parameters. The general structure of observer-based estimator and upper bounds of the estimation errors are derived. On this basis, an optimization tuning procedure for calculation of estimator parameters is proposed. The proposed method is applied for estimation the kinetics of limiting substrates of two real bioprocesses for gluconic acid and alpha-amylase production. The obtained estimates are included in algorithms for control of these processes. The new formalization is applied as well for estimation of ethanol production rate during continuous fermentations with immobilized yeasts. Finally, the paper concludes with some comments concerning the applicability of the proposed software sensor.

II. New Method for Monitoring the Kinetics of On-Line Measured Variables

II.1. New Formalization of Kinetics

The General Dynamical Model (GDM) of biotechnological processes carried out in stirred tank reactor is presented by the following model [1]:

$$\frac{d\boldsymbol{\xi}}{dt} = \mathbf{K}\boldsymbol{\varphi}(t) - D\boldsymbol{\xi} + \boldsymbol{F} - \boldsymbol{Q} \quad (1)$$

where $\boldsymbol{\xi}$ is vector of process state variables (concentrations of components of liquid); $\mathbf{K}$ – matrix of yield coefficients; $\boldsymbol{\varphi}(\tau)$ – vector of reaction rates including unknown time-varying parameters; $\boldsymbol{D}$ – dilution rate; $\boldsymbol{F}$ – vector of mass feed rates in the reactor, $\boldsymbol{Q}$ – vector of rates the mass outflow of the components $\boldsymbol{\xi}$ from the reactor in gaseous form.

It is assumed that:

A1. Each measured component of the liquid phase is related to one kinetic term only, which is considered as unknown time-varying parameter.

A2. The transport dynamics is known.

Under assumptions A1 and A2, GDM is presented as

$$\frac{d\boldsymbol{\xi}_{\boldsymbol{m}}}{dt} = \boldsymbol{\phi}_{\boldsymbol{m}} - D\boldsymbol{\xi}_{\boldsymbol{m}} + \boldsymbol{F}_{\boldsymbol{m}} - \boldsymbol{Q}_{\boldsymbol{m}} \quad (2)$$

where $\boldsymbol{\xi}_{\boldsymbol{m}} \in \mathbb{R}^{n_m x1}$ is vector of concentrations of measurable variables with dimension n_m; $\boldsymbol{\phi}_{\boldsymbol{m}}$ – vector of unknown time-varying parameters describing kinetics of these variables with dim(n_m); $\boldsymbol{F}_{\boldsymbol{m}} \in \mathbb{R}^{n_m x1}$ – mass feed rates in the reactor, $\boldsymbol{Q}_{\boldsymbol{m}}$ – vector of rates the mass outflow of the components

$\boldsymbol{\xi}_m$ from the reactor in gaseous form with dimension n_m. Vectors from equation (2) are sub-vectors of corresponding ones from equation (1).

II.2. Structure of General Software Sensor of Measurable Variable's Kinetics – Stability Analysis

The following structure is proposed:

$$(3)\quad \frac{d\hat{\boldsymbol{\xi}}_m}{dt} = \hat{\boldsymbol{\phi}}_m - D\boldsymbol{\xi}_m + \boldsymbol{F}_m - \boldsymbol{Q}_m + \boldsymbol{\Omega}(\boldsymbol{\xi}_m - \hat{\boldsymbol{\xi}}_m)$$

$$(4)\quad \frac{d\hat{\boldsymbol{\phi}}_m}{dt} = \boldsymbol{\Gamma}(\boldsymbol{\xi}_m - \hat{\boldsymbol{\xi}}_m)$$

where $\hat{\boldsymbol{\phi}}_m$ are the estimates of unknown time-varying parameters, $\boldsymbol{\phi}_m$, describing kinetics of state variables $\boldsymbol{\xi}_m$; $\hat{\boldsymbol{\xi}}_m$ – the estimates of $\boldsymbol{\xi}_m$; $\boldsymbol{\Omega}$ and $\boldsymbol{\Gamma}\in\mathbb{R}^{n_m\,xn_m}$ are matrices, whose elements are the tuning parameters of estimator.

Stability analysis

It is realized under the following realistic assumptions:

A3. Measurements of concentrations $\boldsymbol{\xi}_m$ are corrupted by additive noise vector $\boldsymbol{E}(t)$

$$(5)\quad \boldsymbol{\xi}_{mn}(t) = \boldsymbol{\xi}_m(t) + \boldsymbol{E}(t)$$

where $\boldsymbol{E}(t)=|\,\varepsilon_1, \varepsilon_2, \ldots, \varepsilon_i \ldots, \varepsilon_{nm}\,|^T$ is vector with dim (n_m).

A4. Kinetics term $\boldsymbol{\phi}_m$ is bounded

$$(6)\quad 0 < \boldsymbol{\phi}_m(t) < \boldsymbol{\phi}^* \qquad \forall t$$

where $\boldsymbol{\phi}^*=|\,\boldsymbol{\phi}^*_1, \boldsymbol{\phi}^*_2, \ldots, \boldsymbol{\phi}^*_i \ldots, \boldsymbol{\phi}^*_{nm}\,|^T$ is vector with dim(n_m).

A5. The time-derivative of $\boldsymbol{\phi}_m(t)$ is bounded

$$(7)\quad |d\boldsymbol{\phi}_m / dt| \le \boldsymbol{M}_1 \quad \forall t$$

where $\boldsymbol{M}_1 = |\,m_{11}, m_{12}, \ldots, m_{1i} \ldots, m_{1nm}\,|^T$ is vector with dim(n_m).

A6. The measurements noise is bounded

$$(8)\quad |E(t)| \le \boldsymbol{M}_2 \qquad \forall t$$

where $\boldsymbol{M}_2 = |\,m_{21}, m_{22}, \ldots, m_{2i} \ldots, m_{2nm}\,|^T$ is vector with dim(n_m).

A7. The dilution rate is known and bounded

$$(9)\quad 0 \le D(t) \le D_{max} \quad \forall t.$$

Under these assumptions the estimator (3-4) is rewritten as follows:

$$(10)\quad \frac{d\hat{\boldsymbol{\xi}}_m}{dt} = \hat{\boldsymbol{\phi}}_m - D\boldsymbol{\xi}_{mn} + \boldsymbol{F}_m - \boldsymbol{Q}_m + \boldsymbol{\Omega}(\boldsymbol{\xi}_{mn} - \hat{\boldsymbol{\xi}}_m);$$

$$(11)\quad \frac{d\hat{\boldsymbol{\phi}}_m}{dt} = \boldsymbol{\Gamma}(\boldsymbol{\xi}_{mn} - \hat{\boldsymbol{\xi}}_m).$$

Consider a single inpit–singe output (SISO) system where the vectors $\boldsymbol{\xi}_m$, $\boldsymbol{F}_m$, $\boldsymbol{\phi}_m$, $\boldsymbol{M}_1$, $\boldsymbol{M}_2$ and matrices $\boldsymbol{\Omega}$ and $\boldsymbol{\Gamma}$ in equations (10)-(11) are the scalar quantities: $\boldsymbol{\xi}_m(t)=\boldsymbol{\xi}_1$, $\boldsymbol{F}_m=F_1$, $\boldsymbol{\phi}_m(t)=\boldsymbol{\phi}_1$, $\boldsymbol{E}=\boldsymbol{\varepsilon}_1$, $\boldsymbol{M}_1=m_{11}$, $\boldsymbol{M}_2=m_{21}$, $\boldsymbol{\Omega}=\omega_1$ and $\boldsymbol{\Gamma}=\gamma_1$. Defining the errors $\tilde{\xi}_1 = \xi_1 - \hat{\xi}_1$ and $\tilde{\phi}_1 = \phi_1 - \hat{\phi}_1$ the following system is derived from Eqs. (10)-(11):

$$(12)\quad \frac{d\boldsymbol{x}}{dt} = \mathbf{A}\boldsymbol{x} + \boldsymbol{u}$$

with $\boldsymbol{x} = \begin{vmatrix}\tilde{\xi}_1\\ \tilde{\phi}_1\end{vmatrix}$, $\mathbf{A} = \begin{vmatrix}-\omega_1 & 1\\ -\gamma_1 & 0\end{vmatrix}$, $\boldsymbol{u} = \begin{vmatrix}(D-\omega_1)\varepsilon_1\\ -\gamma_1\varepsilon_1 + \dfrac{d\phi_1}{dt}\end{vmatrix}$.

Let λ_1 and λ_2 be eigenvalues of matrix **A** related to ω_1 and γ_1 as follows:

$$(13)\quad \omega_1 = -(\lambda_1 + \lambda_2); \qquad g_1 = \lambda_1\lambda_2.$$

Asume that:

A8. Design parameters ω_1 and γ_1 are chosen such that matrix **A** has real distinct eigenvalues

$$(14)\quad \lambda_2 < \lambda_1 < 0; \qquad \gamma_1 \le \omega_1^2/4.$$

The following stability results are derived:

Theorem. Under assumptions A1–A8, the estimation errors $\tilde{\xi}_1$ and $\tilde{\phi}_1$ are bounded for all t and asymptotically bounded as follows:

$$(15)\quad \limsup_{t\to\infty} |\tilde{\xi}_1(t)| \le \frac{2m_{21}\delta\beta_{11}}{\sqrt{\omega_1^2 - 4\gamma_1}} + \frac{\beta_{21}}{\gamma_1};$$

$$(16)\quad \limsup_{t\to\infty} |\tilde{\phi}_1(t)| \le m_{21}\beta_{11} + \omega_i\frac{\beta_{21}}{\gamma_1}$$

with $\beta_{11} = D+\omega_1$ and $\beta_{21} = m_{21}\gamma_1 + m_{11;}$

$$\delta = \left(\frac{\lambda_1}{\lambda_2}\right)^{\lambda_1/\lambda_1-\lambda_2} - \left(\frac{\lambda_1}{\lambda_2}\right)^{\lambda_2/\lambda_1-\lambda_2}.$$

The proof is given in [10] as well as the corresponding stability and convergence analysis for MIMO system. The derived upper bound from Eq. (16) is at the root of proposed tuning procedure of the estimator's parameters.

II.3. Tuning Procedure

It follows the idea that as an optimal value of the estimator parameter ω_i can be considered this one minimizing the asymptotic upper bound of $\tilde{\phi}_i(t)$ from Eq. (16) defined as follows:

(17) $\omega_{i\,opt} = \arg\min_{\omega_i} (\limsup_{t\to\infty} \left|\tilde{\phi}_{ui}(t)\right|)$.

In results the following expression is derived:

(18) $$\omega_{iopt} = 2\zeta\sqrt{\frac{m_{1i}}{2m_{2i}}}.$$

The optimal value of the other parameter γ_i is calculated using the relationship between ω_i and γ_i from Assumption A8 presented as follows:

(19) $$\gamma_{iopt} = \omega_{iopt}^2 / 4\zeta^2$$

where ζ is dampling coefficient which value is fixed close to 1 according common engineering rule of thumb.

III. Applications

III.1. Control of Gluconic Acid Production by Aspergillus Niger

Biochemical model

The fermentation mechanism of gluconic acid production by *A. Niger* can be represented by the following reaction scheme:

(20) $$\begin{array}{l} S \xrightarrow{\varphi_1} X \\ S + O_2 \xrightarrow{\varphi_2} P \end{array}.$$

The first reaction represents the fermentative growth of biomass, *X*, which is the catalyst of this reaction. The second reaction represents the conversion of glucose, *S*, to gluconic acid, *P*. With φ_1 and φ_2 are denoted biomass growth and gluconic acid production rates respectively.

The biochemical model proposed in [11] is described by the following equations:

(21) $$\frac{dX}{dt} = \varphi_1;$$

(22) $$\frac{dS}{dt} = -k_1\varphi_1 - k_2\varphi_2;$$

(23) $$\frac{dO_2}{dt} = -k_3\varphi_2 + K_L a(O_2^* - O_2);$$

(24) $$\frac{dP}{dt} = \varphi_2$$

where

(25) $$\varphi_1 = \mu_{\max} X \frac{k - X}{k};$$

(26) $$\varphi_2 = \mu_p P \frac{(k_p - P)}{k_p}$$

with O_2 – concentration of dissolved oxygen in culture medium; $K_L a$ – saturation constant; k, k_1-k_3, μ_{max}, μ_p – kinetic constants.

The model (21)-(26) parameters are estimated using experimental data of the process on the basis of optimization algorithm. This model fits well with the data and is applied as object during simulation investigations of derived below estimation and control algorithms. For their design, so called "operational models" have to be developed. They have to describe the dynamics of the main process variables with high accuracy.

Operational model of process

From the practical experience, it is known that the concentration of target product, gluconic acid, could be increased by applying continuous mode of cultivation where the limiting substrate, glucose, has to be maintained at a previously fixed low value, S^*. To that purpose, a operational model of the process under consideration has to be derived applying the General Dynamical Model Approach [1]. This model is derived on the basis of the reaction scheme (20) as follows:

(27) $$\frac{d}{dt}\begin{bmatrix} X \\ S \\ O_2 \\ P \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ -k_1 & -k_2 \\ 0 & -k_3 \\ 0 & 1 \end{bmatrix} \begin{bmatrix} \varphi_1 \\ \varphi_2 \end{bmatrix} - D\begin{bmatrix} X \\ S \\ O_2 \\ P \end{bmatrix} + \begin{bmatrix} 0 \\ DS_{in} \\ K_L a(O_2^* - O_2) \\ 0 \end{bmatrix}.$$

The right hand side of model (27) consists of two main parts. The first term describes the process kinetics while the other terms refer to the transport dynamics. To be controlled measured varibles glucose, its kinetics has to be known on-line. According the model (27), the glucose kinetics is presented as $\phi_S(t) = -k_1\varphi_1(t) - k_2\varphi_2(t)$ and its estimation requires exact information for the parameters in the right side of equation. Even the reaction rates φ_1 and φ_2 to be estimated correctly, the values of yield coefficients k_1 and k_2 differ from batch to batch processes due to the lack of experiment's reproductivity. Applying the new formalization, the kinetics of measured variable glucose is considered as fully unknown, time-varying parameter $\phi_s(t)$ and estimated by proposed method [12].

Software sensor of limiting substrate's kinetics

The software sensor (3-4) is written in the form

(28) $$\frac{d\hat{S}}{dt} = \hat{\phi}_s - DS_m + DS_{in} + \omega(S_m - \hat{S});$$

(29) $$\frac{d\hat{\phi}_s}{dt} = \gamma(S_m - \hat{S})$$

where $\boldsymbol{\xi_m}=S$, $\boldsymbol{\phi_m}(t)=\phi_s$, $\boldsymbol{\xi_{mn}} = S_m = S + \varepsilon_1$, $\boldsymbol{F_m}=DS_{in}$.

The quality of the estimation is investigated by numerical simulations where the estimator is included in the structure of adaptive linearizing control algorithm shown in *figure 1*.

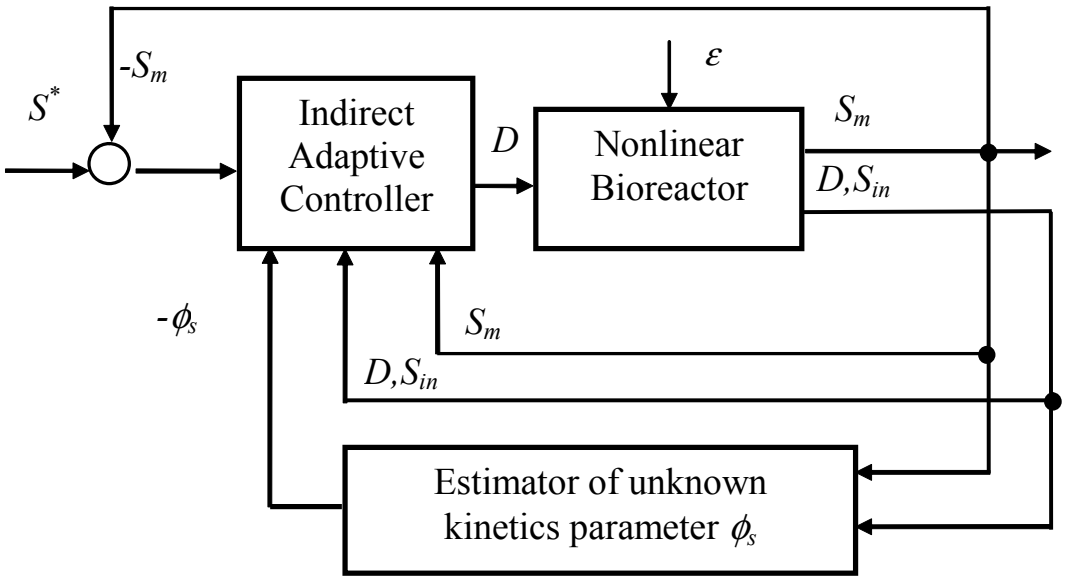

Figure 1. Process control scheme

Results and discussions

The simulations are performed under the following conditions: Fermentation is started as batch one. The initial values of the variables model (21)-(26) are: $X(0)$=0.1[g/l]; $S(0)$= 0.01[g/l]; $P(0)$= 0.01[g/l], $O_2(0)$= 0.0075 [g/l]. The initial value $\hat{S}(0)$ from Eqn. (28) is taken as the model one, while the initial value of $\hat{\phi}_S$ is calculated on the basis of relationship $\phi_S(t) = -k_1\varphi_1 - k_2\varphi_2$ using the initial values of the variables X and P in Eqs. (21) and (24). When the glucose concentration reaches the set point, S^*, the control is turn on. Low values of the set point (between 1 and 3 [g/l]) are recommended because of the inhibition effect of high glucose concentration in the reactor. The value of glucose concentration in the feed, S_{in}, is constant and equal to 200 [g/l]. The performance of the control algorithm is evaluated by combining the following conditions: glucose measurements are corrupted with noise, step-changed of set-point (S^*) and different values of control algorithm parameter λ. In all the simulations the design parameters ω and γ are calculated using equations (17) and (19), respectively. In these equations, the upper bound of time-derivative of $\phi_s(t)$, m_{11}, is set to 1. This is the maximum value of the this time-derivative calculated using the model (21)-(26), and derivators of the two reaction rates. A white noise signal, ε_1, is added to model values of glucose concentration to simulate measurement noise at standard deviation 10%. The upper bound of this signal, m_{21}, is taken 0.1. For the considered case, the calculated optimal values of ω and γ are: $\gamma_{opt}^{10\%} = 5$ and $\omega_{opt}^{10\%} = 4.5$. The influence of the control design parameter λ, is illustrated comparing the results obtained with two different values.

The simulations are carried out with step-changed set point, S^*, from 1 to 2 and from 2 to 3 [g/l]. The changes of set point value are made after the transient process. The obtained results are shown in *figures 2* and *3*. *Figure 2* illustrate the effect of D on the control output (S). The results show that the calculated optimal values of estimator design parameters, ω and γ, guarantee asymptotical tracking error of control output decreases (see *figure 2b*). As it is expected, the higher the value of λ, the faster the convergence speed of tracking error to zero (see *figure 2b*) as a result of higher control action. Finally, the kinetics estimate, $\hat{\phi}$ and estimation error, $\tilde{\phi}$, are shown in *figures 3a* and *3b*, respectively, where it can be seen that this approach guarantee negligible influence of estimation errors, $\tilde{\phi}$. In *figure 3a* the sensor ability to estimate the substrate consumption rate with good accuracy is demonstrated. In *figure 3b* the estimation error is presented. Its maximal relative value reaches to 8% at the process beginning, where the time derivative value of consumption rate is highest one. After that the error is changed proportionaly to that derivative and after 70 hour is commeasured with glucose measurement error.

Investigations applying step-changed set point underline the results described above.

These simulations perfectly agree with the expected qualitative behavior of the closed-loop system and thus validate the proposed approach. It is noted good agreement between the estimates $\hat{\phi}$ and their "true" values ϕ issued from model simulation. Moreover, these results clearly highlight the readily practical implementation of the proposed algorithm.

As it is shown in the stability analysis, $d\phi_s/dt$ is one of the disturbances in the dynamics of the closed–loop system. This fact explains the large error during the batch phase (*figure 3b*) where the process kinetics changes very fast and its time-derivative has high values.

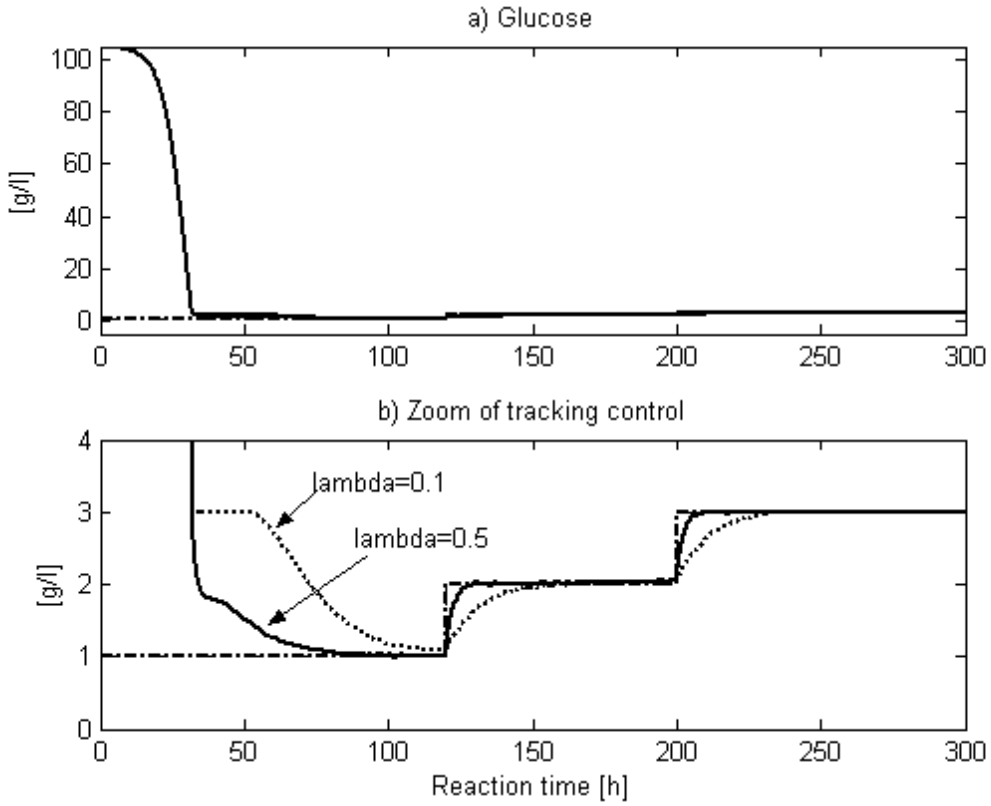

Figure 2. Time-elapse of control output at step changed set point (dash-dot line) from 1 to 3 *g*/*l*. In figure 2*b*, λ=0.1 dotted line, λ=0.5 – solid line

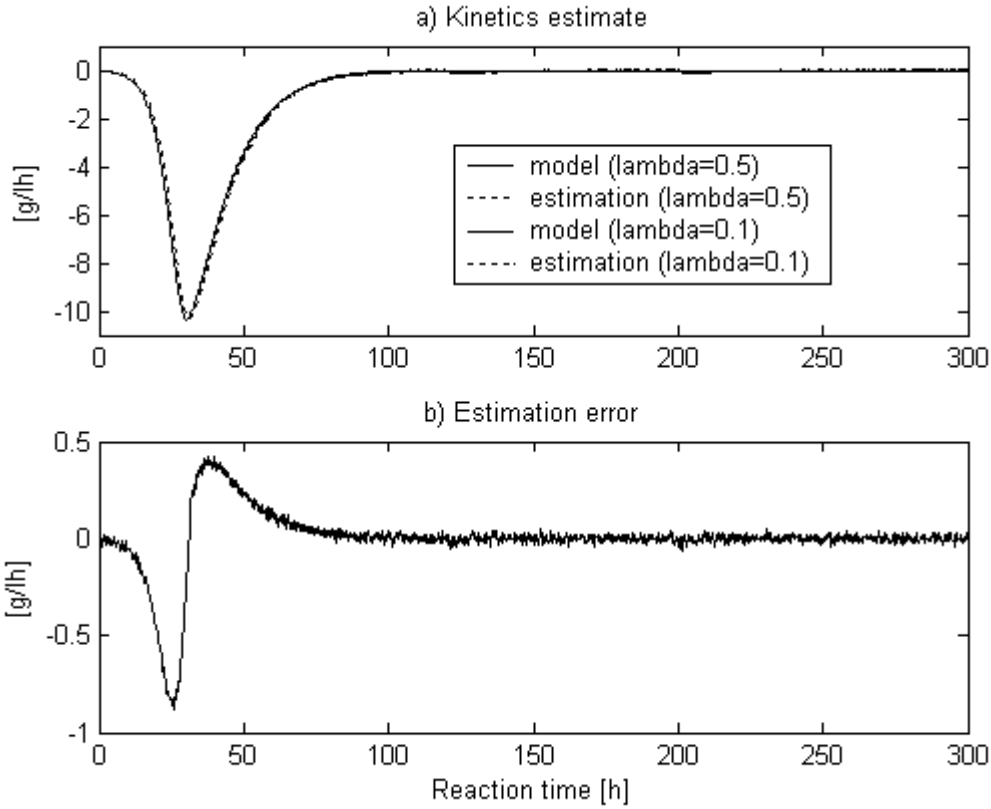

Figure 3. Time-elapse of ϕ_s estimation (a) and error (b) at step changed set point.
Simulations of model (21 –26) extended with transport dynamics (solid lines) are compared with these ones of estimator (dashed line). Simulations with λ=0.5 and λ=0.1 coincide

III.2. Control of α-amylase Production by Bacillus Subtilis

Bacillus species, such as *Bacillus subtilis*, are widely used commercial producers of enzymes and biopharmaceuticals, thanks to their ability to secrete large quantities of proteins directly into culture broth. One of the most effective methods to their production is high cell-density cultivation. Using fed-batch strategy, the high cell density could be attained by avoiding both substrate and metabolite inhibition. Below, the method proposed in Section III.1 is applied for the control of fed-batch production of a-amylase by a recombinant strain of *B. subtilis*.

Biochemical model of the process

The biochemical model for this process is presented in the following equations [13]:

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

$$\frac{dS}{dt} = -X\left(\frac{\mu}{Y_{XS}} + m_S\right) - \frac{F}{V}(S - S_{in})$$

$$(30) \quad \frac{dP_1}{dt} = \alpha_1 \mu X + \beta_1 X - \frac{F}{V}P_1 \quad ;$$

$$\frac{dP_2}{dt} = \alpha_2 \mu X + \beta_2 X - \frac{F}{V}P_2$$

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

where X is biomass concentration in *g/l*, S the glucose concentration in *g/l*, S_{in} the glucose concentration in the feed in *g/l*, P_1 the α-amylase concentration in *g/l*, P_2 the proteinase concentration in *g/l*, V the reactor volume in *l*, F the glucose feed rate in *g/h*, Y_{XS} the biomass/glucose yield coefficient g/g, m_S the maintenance coefficient, α_1, α_2, β_1 and β_2 are kinetic parameters, and μ is the specific growth rate h^{-1}. The specific growth rate is a function presented by Monod's law

$$(31) \quad \mu = \frac{\mu_{\max} S}{K_S + S}$$

where μ_{max} is the maximal value of specific growth rate and K_S the saturation constant.

Parameter identification of the model (30-31) was realized using experimental data from the batch phase of the fermentation by applying an optimization procedure [13]. Since the main control purpose is to stabilize the glucose concentration, a software sensor of its kinetics $\phi_s(t)$ has to be derived.

Software sensor of glucose consumption rate

Following the proposed method for monitoring of unknown kinetics, the following estimator of ϕ_s is derived:

$$(32a) \quad \frac{d\hat{S}}{dt} = \hat{\phi}_s - F(S_m - S_{in}) + \omega_1(S_m - \hat{S}) \; ;$$

$$(32b) \quad \frac{d\hat{\phi}_s}{dt} = \gamma_1(S_m - \hat{S})$$

where the parameters ω_1 and γ_1 are calculated using the expression (17) and (19).

Indirect adaptive linearizing control algorithm

The glucose concentration has to be stabilized at a low value (around 0.3 *g/l*) as by expert's point of view, the production of α-amylase vs. proteinase is most favorable at low glucose concentrations. Applying the indirect adaptive linearizing control low proposed in [1], the following algorithm is derived for feed rate under the considered case:

$$(33) \quad F = \frac{V(\lambda(S^* - S_m) - \hat{\phi}_s)}{(S_{in} - S_m)}$$

where λ is the control tuning parameter, S^* – the set-point of the glucose concentration, $\hat{\phi}_s$ – the estimated value of glucose consumption rate calculated by the observer-based estimator (32).

Simulation investigations

Investigations of the control algorithm (33) is realized by simulations. The model (30), (31) was used as the control plant. Simulated values of glucose concentration were corrupted by additive noise (ε). This is a white noise signal that simulates measurement noise at a standard deviation of 10% of the current S values. Both of the upper bounds m_1 and m_2 in the expressions of estimator's tuning parameters were calculated using the model. The upper bound of time-derivative of $\phi_s(t)$ is m_1=25. The upper bound of the noise signal, m_2, gives the value 0.4. With these values, design parameters ω_1 and γ_1 receive the following ones: $\omega_{1opt}^{10\%} = 11$ (1/h) and $\gamma_{1opt}^{10\%} = 30$ (1/h²). The value of the control design parameter, λ=10, was chosen taking into account the trade off between convergence speed and noise sensibility of the control system.

Results and discussion

The parameter identification results are shown in [13]. The good fits between experimental and model data is the reason the last ones to be applied as real plant in the simulation investigations of algorithm (33). The results are shown in *figure 4*. They are compared with experimental data. Two simulation investigations are realized. The first is at set point 2 g/l for the glucose concentration (like in the experiment) and the second one is at set point 0.3 g/l. In all figures, the results with set-point 0.3 g/l are presented with dished lines while the results with set-point 2 g/l – with lines. Experimental data are presented with circles or stars. For more detail presentation, the results are shown between 6 and 17 hours of cultivation. *Figure 4a* presents the values of glucose consumption rates. A comparison between the model simulations and estimated values (thin lines) shows that the software sensor follows the $\phi_s(t)$ dynamics quite well. *Figures 4b, 4c* and *4d* present the control output, control input and the production of α-amylase and proteinase, respectively. The feed rate control is turned on when the glucose concentration in the reactor reaches the set values (2 or 0.3 g/l).

The simulation results perfectly agreed with the expected qualitative behavior of the closed-loop system. As can be seen in *figure 4b*, the theoretically chosen 10% measurement noise did not influence the quality of the control output which keeps set values in admissible ranges. The characteristic of the measurement noise was used for software sensor design and has to be complied with the precision of the glucose hardware sensor. This is one of future tasks for practical investigations.

In *figure 4c*, the control inputs for both investigations are shown. In *figure 4d,* the results of alpha amylase and proteinase production are presented. The data of proteinese is multiplied by 5 for better presentation. Simulation results (lines) show that alpha amylase growths faster applying the proposed control input comparing with experimental data (circles) in both cases. Unfortunately, this control could be applied till 14 h of fermentation and have to be stopped because of working volume restrictions. This disadvantage could be overcome by adjusting the tuning parameters of software sensor and control algorithm during the experimental investigations of the control scheme. In this way, a higher concentration of the target product a-amylase could be achieved.

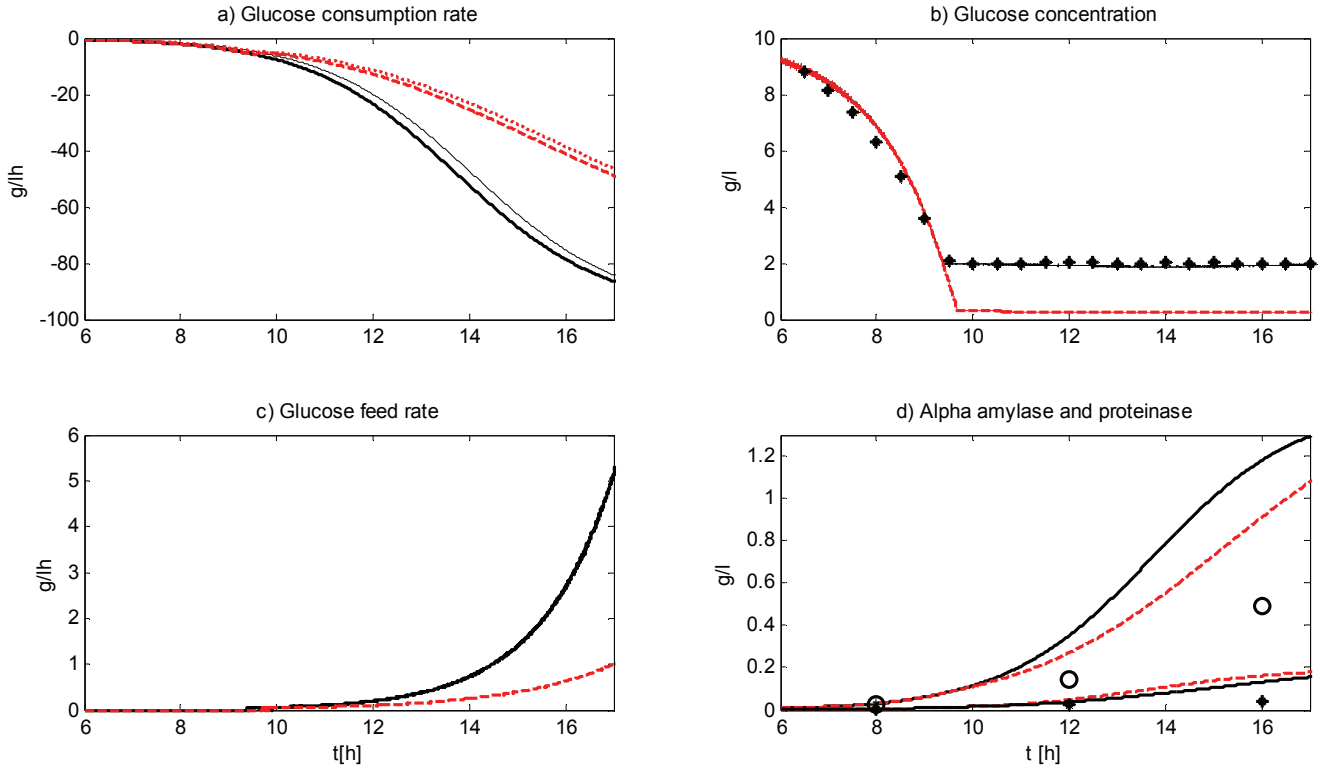

Figure 4. Simulation results

III.3. Monitoring the Ethanol Kinetics During Continuous Fermentation with Immobilized Yeasts Saccharomyces Cerevisiae 46 Evd

The ethanol production is a complex technology, which outputs also a number of secondary products used in various industries. Different research concerning process efficiency increasing by technological refinement of fermentation systems were carried out. Systems with immobilized bio-catalysers with different character and supports' nature were investigated and were compared with traditional fermentation processes with free cells. The comparison is done on the basis of microbial growth kinetics and the metabolite products' accumulation in cultural medium. The systems with immobilized cells showed a number of advantages: high productivity, high operating stability, decreasing of final product purification costs etc. Another contemporary direction for biotechnological processes' efficiency increasing is introduction of on-line control of the main biological variables. Such control schemes can be applied only for continuous or fed-batch cultivation regimes.

During the last years, some results related to monitoring and control of ethanol production processes from recombinant strains *S.cerevisiae* were published [15]. The proposed new control strategies showed increasing of process production and decreasing of production costs. Here an investigation of the new formalization of kinetics is applied for ethanol kinetics estimation for continuous fermentations using experimental data.

Experimental investigations

Ten continuous fermentations with immobilized yeasts *Saccharomyces cerevisiae 46 EVD* are carried out applying ten values of constant dilution rate in the range $0.2[1/h] \leq D \leq 1.5[1/h]$.

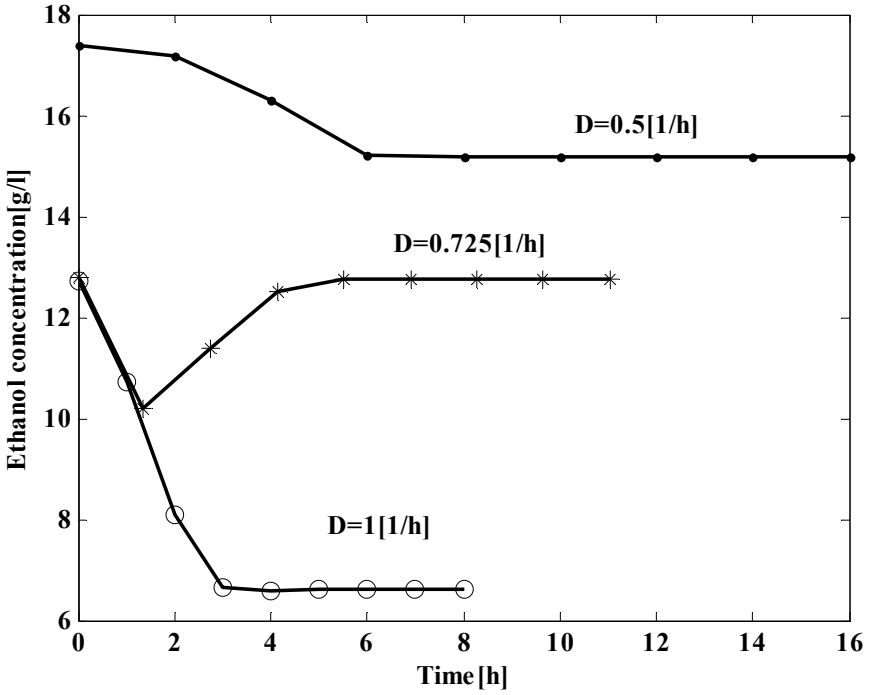

Figure 5. Experimental data of ethanol concentration with D=0.5[1/h] (line with points); D=0.725[1/h] (line with stars) D=1[1/h] (line with circles)

During each experiment nine samples, *k*, are taken at different time intervals dt=1/D[h]. Laboratory measurements of concentration of biomass, *X*, glucose, *S*, and ethanol, *E*, are collected. The ethanol and glucose concentrations were determined by a density meter (Anton Paar DMA 4500, Austria). From experts point of view the fermentations with dilution rates in the range $0.2[1/h] \le D \le 1[1/h]$ are stable and the optimal one as maximal ethanol productivity is realized with D=0.725[1/h]. The experimental values of ethanol – target product during the optimal fermentation and two other fermentations with D=0.5[1/h] and D=1[1/h] are shown in *figure 5*. These three fermentations are used for futher investigation of process monitoring.

Operational model of continuous process

On the basis of the new formalization the following model is proposed:

$$\text{(34a)} \quad \frac{dX}{dt} = \phi_X - DX;$$

$$\text{(34b)} \quad \frac{dS}{dt} = \phi_S - D(S_{in} - S);$$

$$\text{(34c)} \quad \frac{dP}{dt} = \phi_P - DP;$$

where ϕ_X, ϕ_S and ϕ_P are growth rate, substrate consumption rate and ethanol production rate respectively. S_{in}=118.4[g/l] is sugar concentration in the feeding solution during all experiments.

For derivation of control algorithm the value of ethanol production rate, ϕ_P have to be estimated on-line. So, as first task a software sensor of this parameter have to be designed.

Software sensor of ethanol kinetics

The software sensor is based on measurements of ethanol.

Following the general structute Eqs. (3-4), an observer-based estimator for the ethanol production rate, ϕ_P, considered as unknown time-varying parameter, is written as

$$\text{(35a)} \quad \frac{d\hat{P}}{dt} = \hat{\phi}_P - DP + \omega_1(P - \hat{P});$$

$$\text{(35b)} \quad \frac{d\hat{\phi}_P}{dt} = \gamma_1(P - \hat{P}).$$

For the estimator (35), the dynamics of the observation error ($e = P - \hat{P}$) and tracking error ($\rho = \phi_P - \hat{\phi}_P$) is presented by the following system:

$$\frac{d}{dt}\begin{bmatrix} e \\ \rho \end{bmatrix} = \begin{bmatrix} -\omega_1 & 1 \\ -\gamma_1 & 0 \end{bmatrix}\begin{bmatrix} e \\ \rho \end{bmatrix} + \begin{bmatrix} 0 \\ \dfrac{d\phi_P}{dt} \end{bmatrix}.$$

The tuning parameters ω_1 and γ_1 are calculated as function of the eigenvalues, p_o, of this system. To be stable estimator (35), the eigenvalues must lie in the left half plane. The following equations present relationships between the tuning parameters and two eigenvalues p_o selected equal to each other:

$$\text{(36)} \quad \omega_1 = -2p_o \quad \gamma_1 = p_o^2$$

where p_o, ω_1 and γ_1 are constants.

The advantages of the selection of double eigenvalues are well known and described in the literature [10].

Because of the ethanol measurements (*figure 5*), the following discrete time version of the estimator (35) is used

$$\hat{P}(k+1) = \hat{P}(k) + dt[\phi_P(k) - D(k)P(k) + \omega_1(k)(P(k) - \hat{P}(k)]$$
(37)
$$\hat{\phi}_P(k+1) = \hat{\phi}_P(k) + dt[\gamma_1(k)(P(k) - \hat{P}(k)]$$

f the tuning parameters ω_1(k) and γ_1(k) of the discrete estimator are chosen as discrete equivalent of Eq. (36), i.e. constants, it is necessary to check stability conditions – if the eingenvalues of the discrete system lie inside the unit circle. The error system is

$$\frac{d}{dt}\begin{bmatrix} e_{k+1} \\ \rho_{k+1} \end{bmatrix} = \begin{bmatrix} 1-\omega_1 dt & dt \\ -\gamma_1 dt & 1 \end{bmatrix}\begin{bmatrix} e_k \\ \rho_k \end{bmatrix} + \begin{bmatrix} 0 \\ \phi_{P_{k+1}} - \phi_{P_k} \end{bmatrix}.$$

From this system, the discrete eigenvalues p_d are calculated as equal each to other $p_{d1,2}=1+p_o dt$. If $|1+p_o dt|<1$, they fall within the unit circle. This condition is equivalent to

$$-\frac{2}{\Delta t} < p_o < 0 \quad (38)$$

and it is applied to estimator tuning and simulation investigations.

The tuning of the estimator (37) is reduced to finding a proper value for p_o. Since the experiments have been carried out with relatively low frequency (see *figure 5*), and according stability condition (38), maximal eigenvalue, p_{max}, vary in a small interval (see *table 1*). The following criterion is considered concerning the choice of optimal value of tuning parameter

$$Err = min \sum_{k=1}^{N}\left[\frac{E(k) - \hat{E}(k)}{E(k)}\right]^2 \quad (39)$$

where N is number of samples.

Two sets of tuning investigations are realized. The first one concerns experiments with constant dilution rates, shown in *table 1*. The boundaries of eigenvalue p_o for the three experiments are calculated according (38) and optimal values of p_o guarantying minimal mean square errors are calculated from (39). The relative mean estimation errors shown in *table 1* are calculated using the following expression:

$$Err\_rel[\%] = \frac{100}{N}\left[\sum_{k=1}^{N}\left[\frac{E(k) - \hat{E}(k)}{E(k)}\right]\right]. \quad (40)$$

As can be seen, relative mean estimation error increases with increasing of the value of dilution rate. This could be explain with the disturbances at the beginning of the continuous processes due to the change of mode of cultivation from batch (D=0) to continuous.

Table 1. Estimators performance for experiments with different dilution rates

N°	D[1/h]	dt[h]	p_{max}	p_{opt}	Err_rel [%]
1	0.5	2	1	-0.7	1.77
2	0.725	1.379	1.45	-1	4.96
3	1	1	2	-0.6	6.86

The second set of tuning investigations is to compare relative mean estimation errors of process with D=0.725, considered as optimal one with interpolated data of the same process taken under different dt. The aim is to determine a value of dt, leading to acceptable *Err_rel.*

Table 2. Estimator errors with D=0.725

N°	dt[h]	p_{max}	p_{opt}	N° of samples	Err_rel [%]
1	0.1	20	-8	111	0.306
2	0.6896	2.9	-1.7	17	2.224
3	1.379	1.45	-0.9	9	4.960

The results from this investigation are shown in *table 2* and in *figure 6.* As can be expected the relative error, *Err_rel*, increases with increasing of the value of sample time, dt, while upper boundary, p_{max}, and optimal eigenvalue, p_{opt}, decreases. The error at simulation N°2 (dt=0.6896 h) is within the boundary (below 3%), while this one obtained using the experimental data, simulation N°3 (dt=1.379 h) exceed this boundary. As can be seen in *figure 6*, estimates converge to experimental data (subfigures a, b and c) with different rates depending from dt. The ethanol production rate estimates have the same behavour (subfig. d).

IV. Conclusion

In this paper the kinetics monitoring of wide class biotechnological processes carried out in stirred tank reactor is considered. The proposed method is based on new structure of observer-based estimator considering the kinetics of variables as fully unknown time-varying parameters. In such a way the estimation errors arised from constant values of yield coefficients or other kinetic parameters to be avoided. This advantage of new estimator leads to improvement of the kinetics monitoring in comparison with partially known kinetics estimators proposed in the literature. The applications of the method to real bioprocesses prove the theoretical results. Its properties are demonstrated to monitoring and control of other bioprocesses as well [10].

References

1. Bastin, G., D. Dochain. On-line Estimation and Adaptive Control of Bioreactors. Amsterdam, Elsevier Science, 1990.

2. Oliveira, R., E. Ferreira, F. Oliveira, S. Feyo de Azevedo. A Study of the Convergence of Observer-based Kinetics Estimators in Stirred Tank Bioreactors. – *J. Process Control*, 6, 1996, No. 6, 367-371.

3. Oliveira, R., E. C. Ferreira, S. Feyo de Azevedo. Stability, Dynamics of Convergence and Tuning of Observer-based Kinetics Estimators. – *J. Process Control*, 12, 2002, 311–323.

4. Perrier, M., S. Feyo de Azevedo, E. C. Ferreira, D. Dochain. Tuning of Observer-based Estimators: Theory and Application to the On-Line Estimation of Kinetic Parameters. – *Control Eng. Practice*, 8, 2000, 377–388.

5. Biagiola, S., J. Solsona and R. Milocco. Estimation of Kinetic

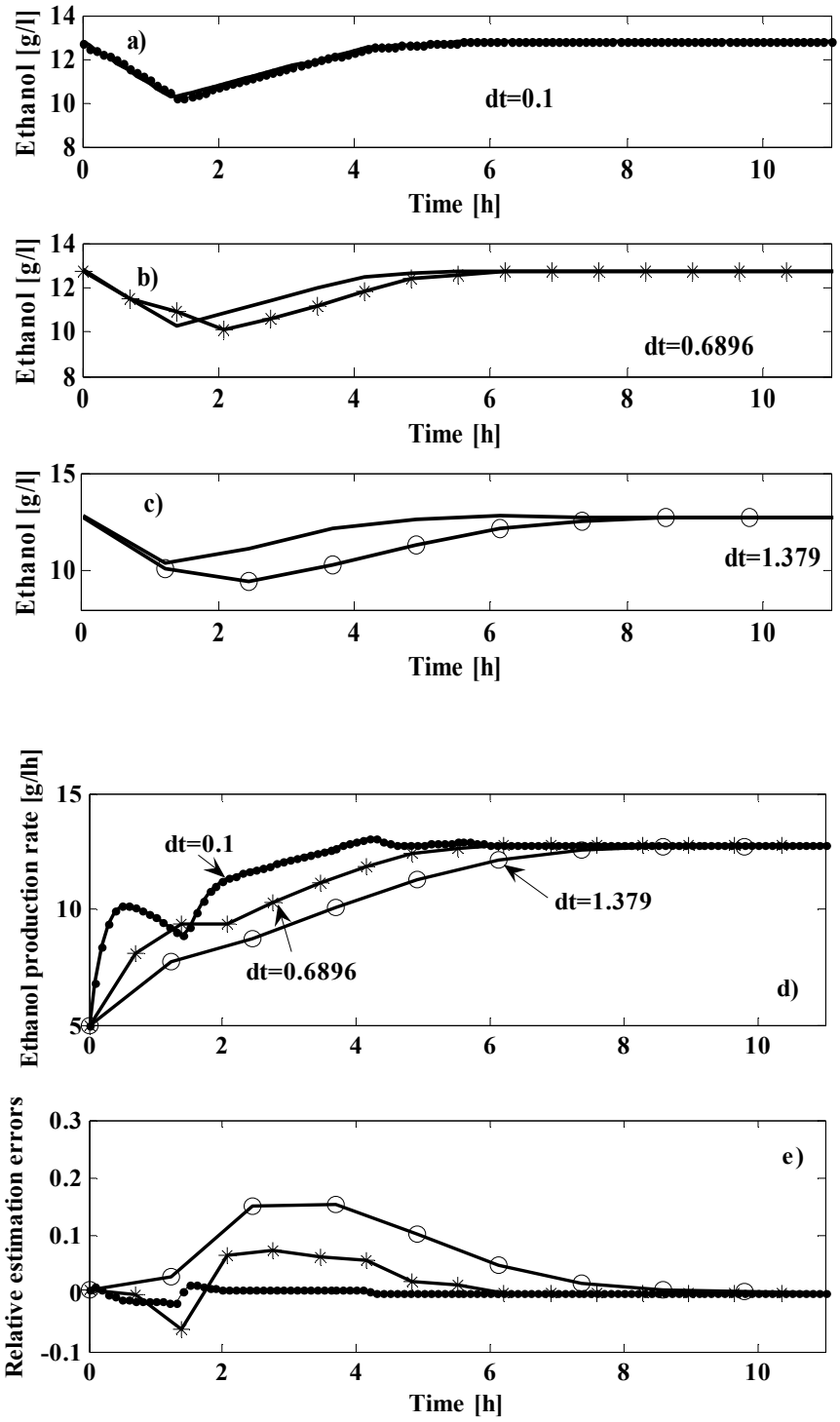

Figure 6. Estimator performance with D=0.725 and different number of samples – estimations of ethanol (subfigures – a, b and c), ethanol production rate (subfigure d) and relative estimation error (subfigure – e)

Rates in Batch Thiobacillus Ferrooxidans Cultures. – *Journal of biotechnology*, 84, 2000, No. 1, 13-25.

6. Claes, J. E. and J. F. Van Impe. On-line Estimation of the Specific Growth Rate Based on Viable Biomass Measurements: Experimental Validation. – *Bioprocess Engineering,* 21, 1999, No. 5, 389-395.

7. Morel, E. et al. On-line Estimation of Kinetic Parameters in Anaerobic Digestion Using Observer-based Estimators and Multi Wave Length Fluorometry. – *Water Science & Technology*, 53, 2006, No. 4-5,77-83.

8. November, E. and J. Van Impe. The Tuning of a Model-Based Estimator for the Specific Growth Rate of Candida Utilis. – *Bioprocess and biosystems engineering*, 25, 2002, No. 1, 1-12.

9. Oliveira, R., R. Simutis and S. Feyo de Azevedo. Design of a Stable Adaptive Controller for Driving Aerobic Fermentation Processes Near Maximum Oxygen Transfer Capacity. – *Journal of Process Control*, 14, 2004, No. 6, 617-626.

10. Lyubenova, V. Monitoring the Kinetics of Biotechnological Processes. DSc Thesis, 2016, Sofia, 216 (in Bulgarian).

11. Ignatova, V., V. Lyubenova, K. Fernandes, M. Garsia, A. Alonso. Model for Control of Gluconic Acid Fermentation by *Aspergillus Niger* and its Application for Observers Design. Proc. Jubilee Conference with International Participation "Agriculture and Food Sciences, Process and Technology", 12-13 May 2005, Sibiu, Romania, 182-189.

12. Ignatova, M., V. Lyubenova, M. R. Garcia, K. V. Fernandes, A. A. Alonso. Indirect Adaptive Linearizing Control of a Class of Bioprocesses – Estimator Tuning Procedure. – *Journal of Process Control*, *Elsevier*, 18, 2008, No. 1, 27-35.

13. Ignatova, M., V. Lyubenova, T. Eerikainen, K. Salonen, K. Kiviharju. Software Sensor of Substrate Kinetics and its Application for Control of α-amylase Production by Bacillus Subtilis. – *Comptes rendus de l'academie bulgare des sciences*, 61, 2008, No. 11, 1449-1458.

14. Angelov, M., G. Kostov. Bioengineering Aspects of Fermentation Processes with Free and Immobilized Microbial Cells, Agency 7D, Plovdiv, Bulgaria, 2011, 201 pages (in Bulgarian)

15. Lyubenova, V., G. Kostov, M. Ignatova. Discrete Adaptive Control of Continuous Fermentation with Immobilized Yeasts Saccharomyces Cerevisiae 46 EVD. Proceed. ECC'12, 2-4 Decembre 2012, Paris, France, 25-29.

Manuscript received on 30.03.2016

Velislava Lyubenova *received MSc degree in Radioelectronics and PhD degree in Bioengineering from Technical University of Sofia in 1987 and 1993 respectively. Since 2000 she is Associate Professor in Institute of Control and Systems Research, Bulgarian Academy of Scieces, now Institute of System Engineering and Robotics. V. Lyubenova received DSc degree in 2016. Her main research activities are in the field of monitoring and control of bioprocesses. She has published more than 70 articles in this field.*

Contacts:
Institute of System Engineering and Robotics
Bulgarian Academy of Science
Acad. G. Bonchev St., bl. 2, P. O. Box 79
tel: (+359 2) 0885504228
e-mail: v_lyubenova@iser.bas.bg