ON – LINE STATE AND PARAMETERS ESTIMATION BASED MEASUREMENTS OF THE GLUCOSE IN MIXED CULTURE SYSTEM

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ABSTRACT

A cultivation model of mixed culture system is used for on-line parameters and cells, lactate, NH_3, poly-β-hydroxybutyrate (PHB) concentrations estimation, based on measurements of the substrate concentration. In this mixed system sugars such as glucose obtained from food processing waste were converted to lactate by Lactobacillius delbrueckii and lactate was converted in turn to poly-β-hydroxybutyrate (PHB) by Ralstonia eutropha in one fermentor as a model system. The proposed procedure is based on the extended Kalman filtering method.

Introduction

Biotechnological processes are dealing with living organisms. Mathematical models with nonlinear differential equations describe them. Real time monitoring of biotechnological processes is very difficult task. There is limited number of sensors capable to provide reliable on-line measurements for such processes. Laboratory and indirect methods determine some of the main variables of biotechnological processes such as biomass and product concentrations. The lack of hardware sensors to perform real time monitoring imposes application of sophisticated methods. This problem is very important in more complex system such mixed system. One decision of the on-line monitoring task is providing procedures for estimation of unmeasurement variables, based of measurement ones.

Mixed or co-culture systems are important for several fermentation processes. There are many fermentation systems, where microorganisms assimilate one substrate and convert it to one metabolite, which is converted from other microorganisms to other metabolite. In our case sugars such as glucose obtained from food processing waste were converted to lactate by *Lactobacillius delbrueckii* and lactate is converted to poly-β-ydroxybutyrate (PHB) by *Ralstonia eutropha* in one fermentor as a model system.

The cell concentration was estimated by measuring the optical density at 660 nm with a spectrophotometer (Ubest-30, Jasco, Tokyo, Japan [10]. The lactate concentration was measured by an enzyme kit (F-kit l-lactic acid 139084, Boehringer Mannheim, Germany) [10]. The ammonium sulphate concentration was measured as the ammonium ion concentration by the enzymatic method (Wako, Osaka, Japan) [10]. The amount of PHB was measured by the improved method of Law and Slepecky [7] using gas chromatograph (GC-8A, Shimadzu, Kyoto, Japan) [5]. The glucose concentration was determined by a calorimetric method using a kit. (Glucose B-test 271-31401, Wako Pure chemical, Osaka, Japan) [10]. When necessary, glucose concentration was measured online using glucose sensor (BF-400, Biot, Tokyo, Japan) with FIA (flow injection analyser system) [10].

The procedure for estimation the difficulty measurable-cells, lactate, amount of PHB and NH_3 concentrations, based on measurements only of one state variable (substrate concentration), is very useful.

Problem statement

For lactic acid fermentation, several mathematical models have been proposed and the dynamical behavior was simulated in the past [1, 6, 7, 11, 12]. In the present study, we extended such models, and considered following model, which can describe the dynamics for cultivation of *L. delbrueckii* based on mass balances with appropriate kinetic expression [10]:

$$\frac{dX_1}{dt} = \mu_1(S,P,DO)X_1 - \frac{F}{V}X_1 \tag{1a}$$

$$\frac{dS}{dt} = -\nu_1(S,P,DO)X_1 + \frac{F(S_F - S)}{V} \tag{1b}$$

$$\frac{dP}{dt} = \sigma_1(S,P,DO)X_1 - \nu_2(S,P,DO)X_2 - \frac{F}{V}P \tag{1c}$$

where μ_1 is specific growth rate of *L. Delbrueckii,* ν_1 is the specific glucose consumption rate and σ_1 is specific lactate production rate.

$$\mu_1(S,P,DO) = \frac{\mu_{m1}(DO)S}{K_S + S}\left(1 - \frac{P}{P_m}\right)^n \tag{2}$$

$$\nu_1(S,P,DO) = \frac{\sigma_1(S,P,DO)}{Y_{P/S}} \tag{3}$$

$$\sigma_1(S,P,DO) = \alpha\mu_1(S,P,DO) + \beta(S,DO) \tag{4}$$

$$\beta(S,DO) = \frac{\beta_m(DO)S}{K_S + S} \tag{5}$$

The second term of the RNS of equations (1c) is due to substrate consumption by *R.eutropha* in the mixed culture and ν_2 was assumed to be of the following form:

$$\nu_2(S,P,DO) = \frac{\mu_2(P,DO,N)}{Y_{X_2/P}(DO)} \tag{6}$$

where $Y_{X2/P}$ is the yield coefficient and was assumed to be a function of DO concentration. μ_2 is the specific growth rate of *R. eutropha,* which be explained next.

For the modelling on *R. eutropha* cultivation, very limited number of papers have been published so far [9, 13]. Applying the mass balances, we considered the following model as [10]:

$$\frac{dX_2}{dt} = \mu_2(S,P,DO)X_2 - \frac{F}{V}X_2 \tag{7a}$$

$$\frac{dN}{dt} = -\nu_3(S,P,DO)X_2 - \frac{F}{V}N \tag{7b}$$

$$\frac{dQ}{dt} = \sigma_2(N)X_2 - \frac{F}{V}Q \tag{7c}$$

where Q is the PHB concentration, σ_2 the specific PHB production rate μ_2 and σ_2 were assumed to be of the following form:

$$\mu_2(P,DO,N) = \frac{\mu_{m2}(DO)P}{K_P + P + P^2/K_i}\left(\frac{N}{K_N + N}\right) \tag{8}$$

$$\nu_3(S,P,DO) = \frac{\mu_2(P,DO,N)}{Y_{X_2/N}(DO)} \tag{9}$$

where μ_2 and σ_2 expresses such experimental phenomena that the cell growth is enhanced as N increases, while PHB production is enhanced as N decreases.

$$\sigma_2(N) = q_m\left(\frac{k_N}{k_N + N}\right) \tag{10}$$

In our work the following values of parameters are used as [10]:

α=1.23 β=1.80 h^{-1} μ_{m1}=0.375 h^{-1}
μ_{m2}=0.734 h^{-1} K_i=2.50 g/l
K_N=0.146 g/l K_P=6.00 g/l
K_S=35.8 g/l n=1 h^{-1}
P_m=42.9 g/l $Y_{P/S}$=0.698
$Y_{X2/N}$=2.41 $Y_{X2/P}$=0.204

State and parameters estimation

Here, the Kalman filtering approach [4] is used for the state under the assumptions that the only substrate is on-line measured.

The adaptive observer can be written in the following form:

$$\frac{d\hat{X}_1}{dt} = \mu_1(\hat{S},\hat{P},D\hat{O})\hat{X}_1 - \frac{F}{V}\hat{X}_1 + w_1(\hat{X}_1,\hat{S},\hat{P},\hat{X}_2,\hat{N},\hat{Q})(S-\hat{S})$$

$$\frac{d\hat{S}}{dt} = -\nu_1(\hat{S},\hat{P},D\hat{O})\hat{X}_1 + \frac{F(S_F-\hat{S})}{V} + w_2(\hat{X}_1,\hat{S},\hat{P},\hat{X}_2,\hat{N},\hat{Q})(S-\hat{S})$$

$$\frac{d\hat{P}}{dt} = \sigma_1(\hat{S},\hat{P},D\hat{O})\hat{X}_1 - \nu_2(\hat{S},\hat{P},D\hat{O})\hat{X}_2 - \frac{F}{V}\hat{P} + w_3(\hat{X}_1,\hat{S},\hat{P},\hat{X}_2,\hat{N},\hat{Q})(S-S) \quad (11)$$

$$\frac{d\hat{X}_2}{dt} = \mu_2(\hat{S},\hat{P},D\hat{O})\hat{X}_2 - \frac{F}{V}\hat{X}_2 + w_4(\hat{X}_1,\hat{S},\hat{P},\hat{X}_2,\hat{N},\hat{Q})(S-\hat{S})$$

$$\frac{d\hat{N}}{dt} = -\nu_2(\hat{S},\hat{P},D\hat{O})\hat{X}_2 - \frac{F}{V}\hat{N} + w_5(\hat{X}_1,\hat{S},\hat{P},\hat{X}_2,\hat{N},\hat{Q})(S-\hat{S})$$

$$\frac{d\hat{Q}}{dt} = \sigma_2(\hat{N})\hat{X}_2 - \frac{F}{V}\hat{Q} + w_6(\hat{X}_1,\hat{S},\hat{P},\hat{X}_2,\hat{N},\hat{Q})(S-\hat{S})$$

$\hat{\xi}^T = \left[\hat{X}_1,\hat{S},\hat{P},\hat{X}_2,\hat{N},\hat{Q}\right]$ denotes the estimates of the state variable vector $\xi^T = [X_1,S,P,X_2,N,Q]$; the measurement variables vector is denoted ξ_1 and is related to the state of the system as follows: $\xi_1 = L\xi$, $\hat{\xi}_1 = L\hat{\xi}$, L = [0 1 0 0 0 0] is a matrix, which select the measured variables.

We choose to estimate parameters k_N and q_m because the others variables don't depends from Q.

The estimation equations of ρ $\rho^T = [q_m, k_N]$, $\hat{\rho}^T = [\hat{q}_m, \hat{k}_N]$ are added to equations of state observer:

$$\frac{dq_m}{dt} = w_7(\hat{X}_1,\hat{S},\hat{P},\hat{X}_2,\hat{N},\hat{Q})(S-\hat{S})$$

$$\frac{dk_N}{dt} = w_8(\hat{X}_1,\hat{S},\hat{P},\hat{X}_2,\hat{N},\hat{Q})(S-\hat{S}) \quad (12)$$

A zero observation error as an equilibrium point of the error model (11, 12) is considered. The observation error is set to govern by the first order differential equation defined by the linear tangent approximation of the equation (11, 12) around $e = 0$:

$$\frac{de}{dt} = A_0(\hat{\xi},\hat{\rho})e \quad (13),$$

where $e = \begin{bmatrix} \xi - \hat{\xi} \\ \rho - \hat{\rho} \end{bmatrix}$, (14)

and A_0 depends on the particular derivatives of the kinetic rates, as follows:

$$A_0(1,1) = \frac{\mu_{m1}S(1-\frac{P}{P_m})}{K_S+S} - \frac{F}{V}$$

$$A_0(1,2) = \frac{\mu_{m1}K_S X_1(1-\frac{P}{P_m})}{(K_S+S)^2} - w_1$$

$$A_0(1,3) = -\frac{\mu_{m1}SX_1}{P_m(K_S+S)}$$

$$A_0(1,4) = 0 \quad A_0(1,5) = 0 \quad A_0(1,6) = 0$$
$$A_0(1,7) = 0 \quad A_0(1,8) = 0$$

$$A_0(2,1) = -\frac{\alpha\mu_{m1}S(1-\frac{P}{P_m}) + \beta_m S}{Y_{P/S}(K_S+S)}$$

$$A_0(2,2) = -\frac{(\alpha\mu_{m1}X_1(1-\frac{P}{P_m}) + \beta_m X_1)K_S}{Y_{P/S}(K_S+S)^2} - \frac{F}{V} - w_2$$

$$A_0(2,3) = \frac{\alpha\mu_{m1}SX_1}{Y_{P/S}P_m(K_S+S)}$$

$$A_0(2,4) = 0 \quad A_0(2,5) = 0 \quad A_0(2,6) = 0$$
$$A_0(2,7) = 0 \quad A_0(2,8) = 0$$

$$A_0(3,1) = \frac{\alpha\mu_{m1}S(1-\frac{P}{P_m}) + \beta_m S}{K_S+S}$$

$$A_0(3,2) = \frac{(\alpha\mu_{m1}X_1(1-\frac{P}{P_m}) + \beta_m X_1)K_S}{(K_S+S)^2} - w_3$$

$$A_0(3,3) = -\frac{\alpha\mu_{m1}X_1S}{P_m(K_S+S)} -$$

$$\frac{\mu_{m2} X_2 N(K_P - \frac{P^2}{K_i})}{Y_{X_2/P}(K_N + N)(K_P + P + \frac{P^2}{K_i})^2} - \frac{F}{V}$$

$$A_0(3,4) = -\frac{\mu_{m2} NP}{Y_{X_2/P}(K_N + N)(K_P + P + \frac{P^2}{K_i})}$$

$$A_0(3,5) = -\frac{\mu_{m2} X_2 PK_N}{Y_{X_2/P}(K_N + N)^2(K_P + P + \frac{P^2}{K_i})}$$

$A_0(3,6) = 0 \quad A_0(3,7) = 0 \quad A_0(3,8) = 0$

$A_0(4,1) = 0 \quad A_0(4,2) = -w_4$

$$A_0(4,3) = \frac{\mu_{m2} X_2 N(K_P - \frac{P^2}{K_i})}{(K_N + N)(K_P + P + \frac{P^2}{K_i})^2}$$

$$A_0(4,4) = \frac{\mu_{m2} PN}{(K_N + N)(K_P + P + \frac{P^2}{K_i})} - \frac{F}{V}$$

$$A_0(4,5) = \frac{\mu_{m2} X_2 PK_N}{(K_N + N)^2(K_P + P + \frac{P^2}{K_i})}$$

$A_0(4,6) = 0 \quad A_0(4,7) = 0 \quad A_0(4,8) = 0$

$A_0(5,1) = 0 \quad A_0(5,2) = -w_5$

$$A_0(5,3) = -\frac{\mu_{m2} X_2 N(K_P - \frac{P^2}{K_i})}{Y_{X_2/N}(K_N + N)(K_P + P + \frac{P^2}{K_i})^2}$$

$$A_0(5,4) = -\frac{\mu_{m2} NP}{Y_{X_2/N}(K_N + N)(K_P + P + \frac{P^2}{K_i})}$$

$$A_0(5,5) = -\frac{\mu_{m2} PX_2 K_N}{Y_{X_2/N}(K_N + N)^2(K_P + P + \frac{P^2}{K_i})} - \frac{F}{V}$$

$A_0(5,6) = 0 \quad A_0(5,7) = 0 \quad A_0(5,8) = 0$

$A_0(6,1) = 0 \quad A_0(6,2) = -w_6 \quad A_0(6,3) = 0$

$$A_0(6,4) = \frac{q_m k_N}{k_N + N} \qquad A_0(6,5) = -\frac{q_m k_N X_2}{(k_N + N)^2}$$

$$A_0(6,6) = -\frac{F}{V} \qquad A_0(6,7) = \frac{k_N}{k_N + N} X_2$$

$$A_0(6,8) = \frac{q_m N X_2}{(k_N + N)^2}$$

$A_0(7,1) = 0 \quad A_0(7,2) = -w_7 \quad A_0(7,3) = 0$

$A_0(7,4) = 0 \quad A_0(7,5) = 0 \quad A_0(7,6) = 0$

$A_0(7,7) = 0 \quad A_0(7,8) = 0 \quad A_0(8,1) = 0$

$A_0(8,2) = -w_8 \quad A_0(8,3) = 0 \quad A_0(8,4) = 0$

$A_0(8,5) = 0 \quad A_0(8,6) = 0 \quad A_0(8,7) = 0$

$A_0(8,8) = 0$ (15)

$\Omega_1 = [w_1 \quad w_2 \ldots \quad w_6]^T$ (16)

$\Omega_2 = [w_7 \quad w_8]^T$ (17)

The gain matrices $\Omega_1(\hat{\xi}, \hat{\rho}), \Omega_2(\hat{\xi}, \hat{\rho})$ are computed in order to minimize the following quadratic criterion:

$$J(t) = \int_0^t \{\frac{d\hat{\rho}^T}{dt}(\tau)\Sigma^{-1}\frac{d\hat{\rho}}{dt}(\tau) + \left\|\xi(\tau) - \hat{\xi}(\tau)\right\|^2\} d\tau, \quad (18)$$

under the constraint of the linear tangent model (13). Σ is weighting matrix.

The solution of this optimisation as follows:

$$R(t) = \begin{bmatrix} R_0(t) & R_1^T(t) \\ R_1(t) & R_2(t) \end{bmatrix},$$

is updated via the following Ricatti equation:

$$\frac{dR}{dt} = -RL^T LR + RA_0^T(\hat{\xi}, \hat{\rho}) + A_0(\hat{\xi}, \hat{\rho})R + \Sigma_0 \quad (19)$$

where L is matrix which selects the measured components;

$$\Sigma_0 = \begin{bmatrix} 0 & 0 \\ 0 & \Sigma \end{bmatrix} \quad (20)$$

The gains Ω_1, Ω_2 are given by:

$$\Omega_1 = [w_1 \quad w_2 \ldots \quad w_N]^T = R_0(t)L^T \quad (21)$$

$$\Omega_2 = [w_{N+1} \ldots \quad w_{N+r}]^T = R_1(t)L^T \quad (22)$$

In our case the matrixes R_0, R_1, R_2 have the following elements:

$$R_0 = \begin{bmatrix} r_1 & r_2 & r_3 & r_4 & r_5 & r_6 \\ r_9 & r_{10} & r_{11} & r_{12} & r_{13} & r_{14} \\ r_{17} & r_{18} & r_{19} & r_{20} & r_{21} & r_{22} \\ r_{25} & r_{26} & r_{27} & r_{28} & r_{29} & r_{30} \\ r_{33} & r_{34} & r_{35} & r_{36} & r_{37} & r_{38} \\ r_{41} & r_{42} & r_{43} & r_{44} & r_{45} & r_{46} \end{bmatrix}$$

$$R_1 = \begin{bmatrix} r_7 & r_{15} & r_{23} & r_{31} & r_{39} & r_{47} \\ r_8 & r_{16} & r_{24} & r_{32} & r_{40} & r_{48} \end{bmatrix}$$

$$R_2 = \begin{bmatrix} r_{49} & r_{50} \\ r_{51} & r_{52} \end{bmatrix} \qquad (23)$$

$$w_1 = r_2, \quad w_2 = r_{10}, \quad w_3 = r_{18}, \quad w_4 = r_{26},$$
$$w_5 = r_{34}, \quad w_6 = r_{42}, \quad w_7 = r_{15}, \quad w_8 = r_{16} \qquad (24)$$

The estimator thus consists of equations (11), (12), (19), and (24). The computation complexity is high due to the need to have the Ricatti equation (19) on-line solved.

The following initial values of the Ricatti matrixes giving satisfactory results are set:

$$R_0 = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 1.3 & 0 & 0 & 0 \end{bmatrix}$$

$$R_1 = \begin{bmatrix} 0 & 0 & 0.138 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix} \qquad (25)$$

As it is stressed in [4] and should be especially noticed here, the stability and convergence properties are extremely difficult to be analysed. Generally speaking, these are still open problems in the case of parameter estimation in nonlinear systems. Extended Kalman filtering estimator may give biased estimates or may even diverge, if it is not carefully initialised.

The estimation of cells Lactobacillus delbrueckii, glucose, lactate, Ralstonia eutropha, NH_3 and poly-β-hydroxybutyrate (PHB) concentrations and parameters are presented in **Fig. 1 to Fig. 8**. Stars denote the experimental data. The models data are given by lines. The estimated data are

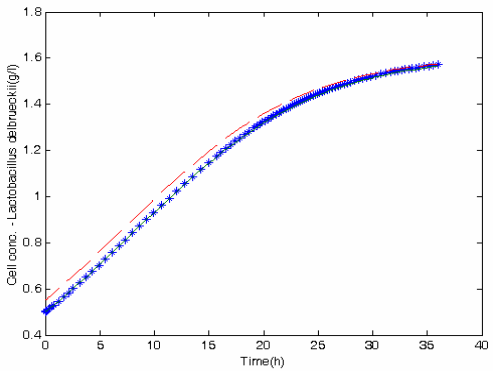

Fig. 1. Cells concentration - *Lactobacillus delbrueckii,* . The model's data - lines. The estimated data - pluses, and estimated data with 5% deviation from initial values - dotted lines.

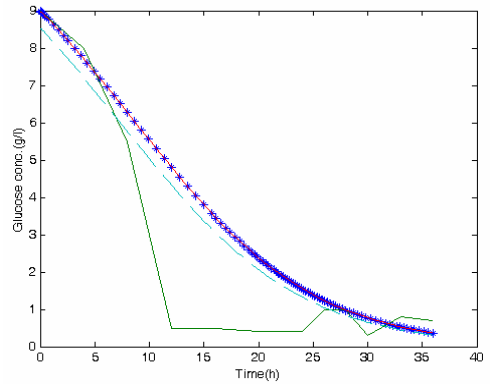

Fig. 2. Glucose concentration, . Experimental data - stars -. The model's data - lines. The estimated data - pluses, and estimated data with 5% deviation from initial values - dotted lines.

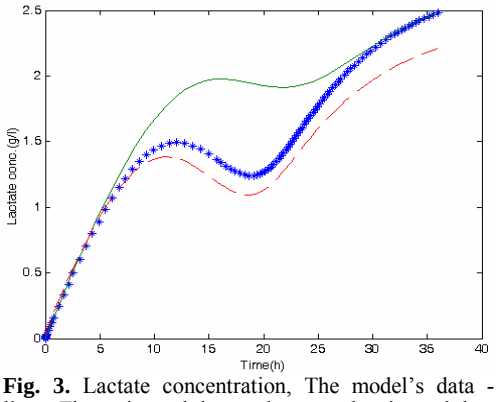

Fig. 3. Lactate concentration, The model's data - lines. The estimated data - pluses, and estimated data with 5% deviation from initial values - dotted lines.

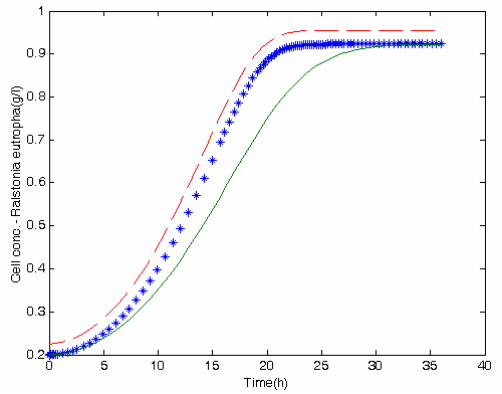

Fig. 4. Cells concentration - *Ralstonia eutropha*, The model's data - lines. The estimated data - pluses, and estimated data with 5% deviation from initial values - dotted lines.

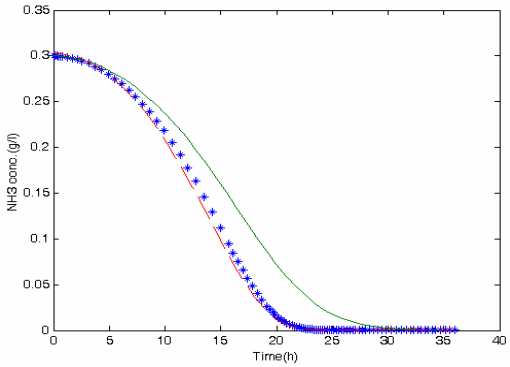

Fig. 5. NH_3 concentration, The model's data - lines. The estimated data - pluses, and estimated data with 5% deviation from initial values - dotted lines.

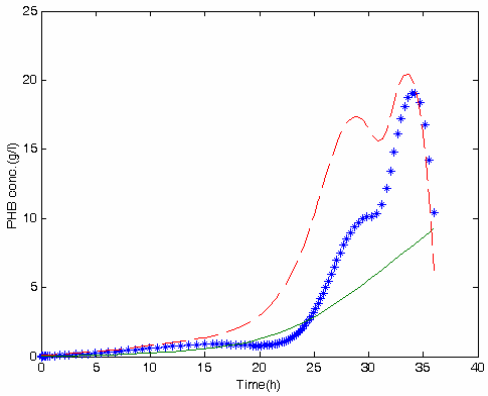

Fig. 6. Poly-β-hydroxybutyrate (PHB) concentrations The model's data - lines. The estimated data - pluses, and estimated data with 5% deviation from initial values - dotted lines.

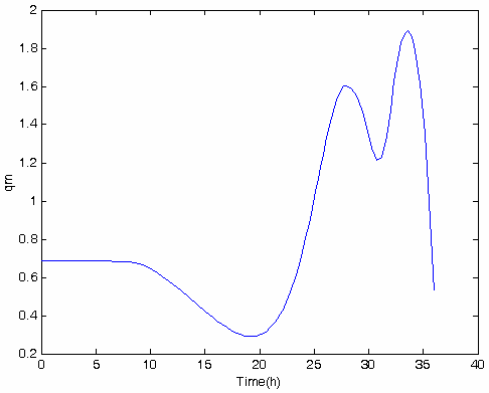

Fig. 7. Estimation of model's parameter q_m.

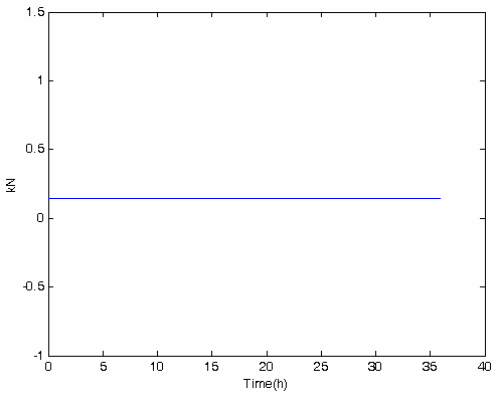

Fig. 8. Estimation of model's parameter k_N.

shown by pluses, and estimated data with 5% deviation from initial values by dotted lines.

Conclusions

A cultivation model of mixed culture system is used for on-line state (cells, lactate and NH_3 and PHB concentrations) and parameters estimation, based on measurements of the substrate concentration. A design procedure based on the extended Kalman filtering method. The simulation investigations carried out under deviations in the initial conditions confirm the applicability of the proposed estimation procedure.

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