

Software Sensors Design for a Class of Aerobic Fermentation Processes

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Abstract: The problem of on-line state and parameter estimation (software sensors design) of a class of aerobic fermentation processes for metabolite product formation is considered. The class is characterized by: two limiting substrates one of which, growth factor, is practically depleted during the biomass growth where the product formation is negligible; corresponding general reaction scheme – a qualitative description of the main metabolic reactions between the main components in the liquid phase (biomass, substrates, product and dissolved oxygen concentrations). Two separate sensors – state and parameter estimators – are designed. The state estimator is developed based on knowledge of only one on-line measurable variable, the dissolved oxygen, and the yield factors assumed as constant coefficients. Parameter estimator of the specific reaction rates is developed under the assumption that all the process variables are known on-line by measurements or estimates. The yield factors are estimated also as non-stationary parameters, thus creating a basis for comparison with the specified constant values used for the state estimator design. As a case study industrial Lysine fermentation in fed-batch mode of operation is considered. Simulation investigations under different operating conditions are done in order to highlight the performances of the proposed sensors.

Keywords: Bioreactors, Aerobic fermentation, Software sensors.

Introduction

The main difficulty in bioreactor control lies in the absence, in most cases, of cheap and reliable instrumentation suited to real time monitoring-sensors, capable of providing on-line measurements of the biological variables (state of the system) required for implementation of high performance automatic control. In case of adaptive model-based control, which is proved to be suitable for this kind of processes, the estimates of the reaction rates and the yield coefficients should be also available. The design of model-based state and parameter estimators (software sensors), providing reliable on-line information for the biological variables and model parameters, for nonlinear systems in general, and particularly for the biotechnological systems, has always been a matter of intense research [1-3, 5-8, 10, 13-16, 18-23]. The process model (sometimes in quite a general form with parameters that should be estimated on-line) plays an important role in the development of the estimation algorithms. Depending on the current knowledge for the concrete process with respect to the kinetics (kinetic rates and yield coefficients) the design can be based on the assumptions for completely known process kinetics, or on the more realistic assumption for partially known or completely unknown kinetics. The available on-line measured variables, the mode of

operation, the possibility to get solution for the particular task, and the quality of estimation that must be achieved play also important role in case of software sensors design.

An opportunity to overcome the need of prior knowledge for the reaction kinetics lies in asymptotic estimators design [2, 5]. The approach is based on a General Dynamic Model (GDM) of the process, developed on the basis of a corresponding General Reaction Scheme (GRS) – a qualitative description of the main metabolic reactions. Such estimators are simpler in comparison with the exponential estimators, based on Kalman filtering method and full knowledge of the process kinetics [1, 6, 7, 14-16]. They are usually used when the process is not exponentially observable, or no hard requirements for the convergence speed exist, as well as especially for the industrial processes, because of the scaling problems.

The process definition by a simple GRS and GDM, and based on them general form of asymptotic estimators [2, 5], create unique possibility for software sensors design, not only for a particular process, but for a class of processes with equal general properties. In this paper, taking into account the main characteristics of a class of *aerobic* fermentation processes for metabolic product formation (specific GRS and process dynamics), two software sensors (state and parameter estimators) are designed. The state estimator is developed based on knowledge of *only one* on-line measurable variable, the dissolved oxygen, and the yield factors assumed as constant coefficients. Parameter estimator of the specific reaction rates is developed under the assumption that all the process variables are known on-line by measurements or estimates. The yield factors are also estimated as non-stationary parameters, thus a basis for comparison with the specified constant values used for the state estimator design is created. As a case study industrial Lysine fermentation in fed-batch mode of operation is considered. Simulation results under different operating conditions are given in order to highlight the performances of the proposed sensors. The sensors are tuned for the process of industrial Lysine production and tested by simulation investigations for fed-batch (and batch – when applicable) mode of operation.

Problem statement

A wide class of aerobic fermentation processes for metabolic production in two limiting substrates media, characterized by the following General Reaction Scheme is considered:



where: a) the process variables (state of the system) are: X – biomass concentration, $[\text{g}\cdot\text{l}^{-1}]$; A – product concentration, $[\text{g}\cdot\text{l}^{-1}]$; S – limiting substrate concentration, $[\text{g}\cdot\text{l}^{-1}]$; R – limiting substrate concentration which determines the growth (growth factor), $[\text{g}\cdot\text{l}^{-1}]$; C – dissolved oxygen concentration, [%]; b) the process parameters are the kinetic rates φ_i ($i = 1, 2$), $[\text{h}^{-1}]$ and the yield factors k_i ($i = 1, 1$), $[\text{g}\cdot\text{g}^{-1}]$, which participate in the General Dynamical Model corresponding to this reaction scheme.

The first path (microbial growth with a negligible production, with kinetic rate φ_1) requires limiting substrates S and R . The second path (enzyme catalysed product formation with kinetic rate φ_2) is determined by the first limiting substrate S , only, since the growth factor R , is completely depleted during the biomass growth. For the sake of generality the product A

will be kept in the first reaction, but in the process of software sensors design it will be taken into account that no significant product formation is presented.

It is also assumed, for the case of state estimator design, that the control input, i.e. the feed rate F (the dilution rate D) is not zero during a long period of time, which means that the results are valid for continuous and fed-batch mode of operation. This assumption is due to the requirements for the convergence of the developed below algorithms [9].

The corresponding general mass-balance model (accounting for the components in the liquid phase only) has the form:

$$\dot{\xi} = K\varphi(\xi) - D\xi + F_{in}, \quad (2)$$

where:

– $\xi \in R^{1 \times 5}$ is state vector of the system:

$$\xi = [X \quad A \quad S \quad R \quad C]^T \quad (3a)$$

– $K \in R^{5 \times 2}$ is matrix of the yield coefficients

$$K = \begin{bmatrix} 1 & 0 \\ k_6 & 1 \\ -k_1 & -k_2 \\ -k_3 & 0 \\ -k_4 & -k_5 \end{bmatrix}; \quad k_6 \approx 0; \quad (3b)$$

– $\varphi(\xi) \in R^{2 \times 1}$ is vector of the kinetic rates

$$\varphi(\xi) = \begin{bmatrix} \varphi_1 \\ \varphi_2 \end{bmatrix}; \quad (3c)$$

– $F_{in} \in R^{1 \times 5}$ is vector of the transfer rates

$$F_{in} = [0 \quad 0 \quad F_{in_s} \quad F_{in_R} \quad Q_{in}]^T = [0 \quad 0 \quad DS_f \quad DR_f \quad Q_{in}]^T; \quad (3d)$$

– $S_f \in R^{1 \times 1}$ and $R_f \in R^{1 \times 1}$ are the concentrations of the limiting substrate and the growth factor in the input flow, respectively;

– $D \in R^{1 \times 1}$ is dilution rate which for the case of fed-batch mode of operation can be also expressed as follows:

$$D = \frac{F}{V}; \quad (3e)$$

– F is the feed rate and V is the volume:

$$\frac{dV}{dt} = F; \quad (3f)$$

– $Q_{in} \in R^{1 \times 1}$ is the oxygen transfer rate.

The aim of the present work is, for the class of aerobic fermentation process under consideration, and in particular for Lysine fermentation:

- a) the possibility for on-line estimation of the main process variables based on on-line measurements of the dissolved oxygen only to be studied;
- b) estimation algorithms (software sensors) for the main process variables and the kinetic parameters (reaction rates and yield coefficients), based on General Reaction Scheme of the process (1) and general mathematical model (2)-(3) to be developed.

As a case study industrial Lysine fermentation (100 m³ bioreactor) with *Brevibacterium flavum* will be considered. A biochemical model [9] of the process will be used for tuning the algorithms, and simulation investigations will be carried out in order to validate the results.

Process state estimation

The problem is to design an asymptotic state estimator (software sensor) of the unknown variables (X, S, R, A), based on on-line measurements of the dissolved oxygen C . Matrix K of the yield coefficients and on-line information for the transfer rates F_{in} and Q_{in} should also be available.

In order to use the proposed method for asymptotic estimators design the number q of measured state variables should be equal to or greater than the rank of the matrix K : $q = \dim(\xi_1) \geq p = \text{rank}(K)$ [2]. Since in our case $\text{rank}(K) = 2$ and the dissolved oxygen C , only is measured on-line, i.e. $q = 1$, the last requirement is not fulfilled.

From the description of the class of processes under consideration, it can be seen that the two biological paths are mutually exclusive, i.e. only one reaction is activated at each time. In this case the process could be divided on two separate phases. They could be easily recognized by the estimates of the growth factor: growth without, or with very small, negligible, production (*Phase A*) and production without growth (*Phase B*). This allows us to consider two different dynamical models for description of each phase (*Model A* and *Model B*).

An other problem that arise in solving the particular task is concerned to the fact that the growth factor can be added trough the input feeding flow. Thus the assumption for complete depletion of the growth factor during the growth phase cannot be used. In order to account for these peculiarities a low level (different than zero) of the growth factor R_{\min} , is assumed to be a basis for different phases recognition. The value of R_{\min} is determined by expert knowledge or previous investigations, taking into account the possible values of R_f . In other words, it is assumed that the growth factor is practically depleted during the biomass growth and it is not added during the process, or added to a certain low level R_{\min} that cannot influence significantly the growth rate. As it has been shown on Fig. 1, for the case of industrial fed-batch Lysine fermentation, where $S_f = 220 \text{ g}\cdot\text{l}^{-1}$ and $R_f = 0.95 \text{ g}\cdot\text{l}^{-1}$, the growth factor added during the feeding does not influence significantly the biomass growth rate [9].

Taking into account the abovementioned considerations, two different models, representing the different phases, are considered.

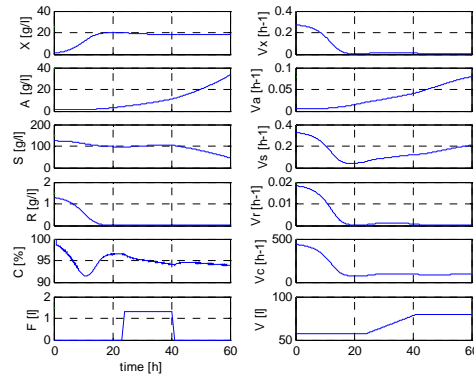


Fig. 1 The main process variables X, A, S, R , the corresponding specific rates V_x, V_a, V_s, V_r

MODEL A – description of the *Phase A* (growth with negligible production), given by (2) where:

$$\xi = [X \ A \ S \ R \ C]^T, \quad (4a)$$

$$K = \begin{bmatrix} 1 \\ k_6 \\ -k_1 \\ -k_3 \\ -k_4 \end{bmatrix}, \quad k_6 \approx 0, \quad (4b)$$

$$\varphi(\xi) = \varphi_1, \quad (4c)$$

$$F_{in} = [0 \ 0 \ DS_f \ DR_f \ Q_{in}]^T \quad (4d)$$

MODEL B – description of *Phase B* (production without growth, in absence, or presence of very limited amount of growth factor, that cannot influence significantly on the growth rate, given by (2) where:

$$\xi = [X \ A \ S \ C]^T, \quad (5a)$$

$$\varphi(\xi) = \varphi_2, \quad (5b)$$

$$F_{in} = [0 \ 0 \ DS_f \ Q_{in}]^T, \quad (5c)$$

$$K = \begin{bmatrix} 0 \\ 1 \\ -k_2 \\ -k_5 \end{bmatrix}. \quad (5d)$$

Thus, the rank of the matrix K of both models is equal to 1, and the measurement of dissolved oxygen only, can be used for the state observation. Now, assuming that:

$$\varphi(\xi) = G(\xi)\alpha. \quad (6)$$

Model (2) can be rewritten in the form:

$$\dot{\xi} = KG(\xi)\alpha - D\xi + F_{in}, \quad (7)$$

where: $\alpha \in R^{2 \times 1}$ is vector of the specific reaction rates (the “unknown part” of the kinetic reaction):

$$\alpha = \begin{bmatrix} \alpha_1 \\ \alpha_2 \end{bmatrix}; \quad (8)$$

$G(\xi) \in R^{2 \times 2}$ is diagonal matrix, presenting the “known” part of the kinetic reaction, the elements of which include the substrates that participate in the corresponding reaction:

$$G(\xi) = \begin{bmatrix} SRCX & 0 \\ 0 & SCX \end{bmatrix}. \quad (9)$$

From the basic properties of the General model (2) it is known that, for the system (2), (4), (6)-(9) (or (2), (5)-(9)) there exist (at least) one state partition (ξ_a, ξ_b) of the state (and the corresponding $K_a, K_b, F_{in_a}, F_{in_b}$ partition), one $(N-p) \times p$ matrix $A_0 = -K_b / K_a$, and one vector Z of dimension $N-p$ defined as the following linear combinations of the state variables:

$$Z = A_0\xi_a + \xi_b \quad (10)$$

and whose dynamics, given by:

$$\frac{dZ}{dt} = -DZ + A_0F_{in_a} + F_{in_b} \quad (11)$$

are independent of the reaction rate $\varphi(\xi)$, where N is the number of the state variables [2].

With appropriate definitions of the $(N-p) \times q$ and $(N-p) \times (N-q)$ matrices A_1 and A_2 , the vector Z can be rewritten explicitly as a linear combination of the vector of the measured variables, ξ_1 , and the vector of unmeasured state variables, ξ_2 :

$$Z = A_1\xi_1 + A_2\xi_2. \quad (12)$$

The design of the estimation algorithm for the *Phase A* and *Phase B* is based on the *Model A* and *Model B*, respectively.

Phase A: ($\hat{R} > R_{\min} > 0$)

The design is based on the *Model A* (7), (4a-4b, 4d) where the vector α and the matrix G are given by:

$$\alpha = \alpha_1, \quad (13a)$$

$$G(\xi) = SRCX. \quad (13b)$$

The following state partition is chosen:

$$\xi_a = C, \quad \xi_b = [X \quad A \quad S \quad R]^T \quad (14a)$$

and

$$K_a = -k_4, \quad K_b = [1 \quad k_6 \quad -k_1 \quad -k_3]^T, \quad (14b)$$

$$F_{in_a} = Q_{in}, \quad F_b = [0 \quad 0 \quad DS_f \quad DR_f]^T. \quad (14c)$$

The matrix K_a is full rank and the partition is admissible. Hence:

$$A_0 = \frac{-K_b}{K_a} = \begin{bmatrix} 1 & k_6 & -k_1 & -k_3 \\ k_4 & k_4 & k_4 & k_4 \end{bmatrix}^T, \quad (15)$$

$$Z = \begin{bmatrix} 1 \\ k_4 \\ k_6 \\ k_4 \\ -k_1 \\ k_4 \\ -k_3 \\ k_4 \end{bmatrix} C + \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} X \\ A \\ S \\ R \end{bmatrix} = \begin{bmatrix} \frac{1}{k_4}C & 1X & 0A & 0S & 0R \\ \frac{k_6}{k_4}C & 0X & 1A & 0S & 0R \\ -\frac{k_1}{k_4}C & 0X & 0A & 1S & 0R \\ -\frac{k_3}{k_4}C & 0X & 0A & 0S & 1R \end{bmatrix}. \quad (16)$$

Since the dissolved oxygen C , is assumed to be the only on-line measurable variable:

$$\xi_1 = C, \quad \xi_2 = [X \quad A \quad S \quad R]^T \quad (17a)$$

and in accordance to (12) and (16) $A_1 \in R^{4 \times 1}$, $A_2 \in R^{4 \times 4}$:

$$A_1 = A_0, \quad A_2 = I_4. \quad (17b)$$

The general form of the asymptotic state estimator is given by [2]:

$$\frac{d\hat{Z}}{dt} = -D + A_0 F_{in_a} + F_{in_b}, \quad (18)$$

$$\hat{\xi}_2 = A_2^+ (\hat{Z} - A_1 \xi_1).$$

The matrix A_2^+ is a left inverse of A_2 . \hat{Z} and $\hat{\xi}_2$ denote on-line estimates of Z and ξ_2 respectively. Taking into account (14)-(17) the process state estimator (observer) for the class of processes under consideration is obtained:

$$\begin{aligned} \frac{d\hat{Z}_1}{dt} &= -D\hat{Z}_1 + \frac{1}{k_4}Q_{in}, \\ \frac{d\hat{Z}_2}{dt} &= -D\hat{Z}_2 + \frac{k_6}{k_4}Q_{in}, \end{aligned} \quad (19a)$$

$$\frac{d\hat{Z}_3}{dt} = -D\hat{Z}_3 - \frac{k_1}{k_4}Q_{in} + DS_f,$$

$$\frac{d\hat{Z}_4}{dt} = -D\hat{Z}_4 - \frac{k_3}{k_4}Q_{in} + DR_f,$$

$$\hat{X} = \hat{Z}_1 - \frac{1}{k_4}C, \hat{A} = \hat{Z}_2 - \frac{k_6}{k_4}C, \quad (19b)$$

$$\hat{S} = \hat{Z}_3 + \frac{k_1}{k_4}C, \hat{R} = \hat{Z}_4 + \frac{k_3}{k_4}C.$$

Phase B: ($R_{min} > \hat{R} > 0$)

The design is based on the *Model B* (7), (5a-5b, 5d), where the vector α and the matrix G are given by:

$$\alpha = \alpha_2, \quad (20a)$$

$$G(\xi) = SCX. \quad (20b)$$

The following state partition is chosen:

$$\xi_a = C, \xi_b = [X \ A \ S]^T, \quad (21a)$$

and

$$K_a = -k_5, K_b = [0 \ 1 \ -k_2]^T, \quad (21b)$$

$$F_{in_a} = Q_{in}, F_{in_b} = [0 \ 0 \ DS_f]^T. \quad (21c)$$

The matrix K_a is full rank and the partition is admissible. Hence:

$$A_0 = \frac{-K_b}{K_a} = \begin{bmatrix} 0 & 1 & -k_2 \\ k_5 & k_5 & k_5 \end{bmatrix}^T, \quad (22)$$

$$Z = \begin{bmatrix} 0 \\ 1 \\ \frac{1}{k_5} \\ -\frac{k_2}{k_5} \\ \frac{1}{k_5} \end{bmatrix} C + \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} X \\ A \\ S \end{bmatrix} = \begin{bmatrix} 0.C & 1.X & 0.A & 0.S \\ \frac{1}{k_5}.C & 0.X & 1.A & 0.S \\ -\frac{k_2}{k_5}.C & 0.X & 0.A & 1.S \end{bmatrix}. \quad (23)$$

Since it is assumed that the dissolved oxygen C , is the only on-line measurable variable,
 $\xi_1 = C$, $\xi_2 = [X \quad A \quad S]^T$ (24a)

and in accordance to (12) and (23):

$$A_1 = A_0, \quad A_2 = I_4. \quad (24b)$$

The process state estimator in this case is found to satisfy the system:

$$\begin{aligned} \frac{d\hat{Z}_5}{dt} &= -D\hat{Z}_5, \\ \frac{d\hat{Z}_5}{dt} &= -D\hat{Z}_5, \\ \frac{d\hat{Z}_6}{dt} &= -D\hat{Z}_6 + \frac{1}{k_5}Q_{in}, \\ \frac{d\hat{Z}_7}{dt} &= -D\hat{Z}_7 - \frac{k_2}{k_5}Q_{in} + DS_f, \end{aligned} \quad (25a)$$

$$\begin{aligned} \hat{X} &= \hat{Z}_5, \\ \hat{A} &= \hat{Z}_6 - \frac{1}{k_5}C, \\ \hat{S} &= \hat{Z}_7 + \frac{k_2}{k_5}C. \end{aligned} \quad (25b)$$

Consequently, the state observer for the class of processes under consideration has the form (19) for the time when $\hat{R} > R_{min} > 0$ and (25) when $R_{min} > \hat{R} > 0$.

The convergence of the algorithm is proved by the theorem, which implies that the dilution rate $D(t)$ does not remain equal to zero for excessively long periods of time [2]. Hence, the convergence of the observer is valid only for fed-batch and continuous operating conditions. The speed of convergence of the estimation is completely determined by the experimental conditions through the value of the dilution rate.

Estimation of the reaction rates and yield coefficients

Software sensor of the kinetics (reaction rates and the yield coefficients), based on on-line knowledge (measurements or estimates) of the process variables and on-line knowledge of the transfer rates, is designed. It will be shown that if the dissolved oxygen C , is omitted from the reaction scheme the accurate estimates of the reaction rates and yield coefficients, could be achieved. It has also done in order to create a basis for comparison of the yield coefficients assumed as constants in case of process state estimator design on the basis of dissolved oxygen measurements only, and the corresponding yield factors (non-stationary parameters) estimated by observer-based estimator, without taking into account the dissolved oxygen. The following reduced reaction scheme can be used for estimator design:



The state space model corresponding to the reaction scheme (26) has the form (2), where:

$\xi \in R^{1 \times 4}$, $K \in R^{4 \times 2}$, $\varphi \in R^{2 \times 1}$, $F_{in} \in R^{1 \times 4}$ and:

$$\xi = [X \quad A \quad S \quad R]^T, \quad (27a)$$

$$K = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ -k_1 & -k_2 \\ -k_3 & 0 \end{bmatrix}, \quad (27b)$$

$$\varphi(\xi) = \begin{bmatrix} \varphi_1 \\ \varphi_2 \end{bmatrix}, \quad (27c)$$

$$F_{in} = [0 \quad 0 \quad F_{in_s} \quad F_{in_R}]^T = DS_{in} = D[0 \quad 0 \quad S_f \quad R_f]^T. \quad (27d)$$

Under the assumption (6) the model (2) can be rewritten in the form (7), where:

$$\alpha = \begin{bmatrix} \alpha_1 \\ \alpha_2 \end{bmatrix}, \quad (28a)$$

$$G(\xi) = \begin{bmatrix} SRX & 0 \\ 0 & SX \end{bmatrix}. \quad (28b)$$

The total number m of unknown yield coefficients in K (denoted as k_1, k_2, \dots) is given by:

$$m = \sum_{j=1}^M (N_j - 1) = 3,$$

where:

- N_j is the number of components involved in the reaction with index j ;
- M is the number of the reactions.

For the class of processes under consideration: $m = 3$. The total number of the unknown parameters is 5 (three yield coefficients and two reaction rates).

The vector $KG(\xi)\alpha \in R^{4 \times 1}$ in (7) may be rewritten in a linear regression form as follows:

$$KG(\xi)\alpha = \begin{bmatrix} \alpha_1 SRX \\ \alpha_2 SX \\ -k_1 \alpha_1 SRX - k_2 \alpha_2 SX \\ -k_3 \alpha_1 SRX \end{bmatrix}. \quad (29)$$

It can be also present in the form:

$$KG(\xi)\alpha = \Phi(\xi)\theta, \quad (30)$$

where:

– $\Phi(\xi)$ is a $N \times (m + M) = 4 \times 5$ matrix of known multilinear combinations of the state variables;

– $\theta^T = [\theta_1 \ \theta_2 \ \dots \ \theta_5]$ is vector of bilinear combinations of the unknown parameters k_i ($i = 1, \dots, 3$) and α_j ($j = 1, 2$), formally written as follows:

$$\theta = f(\alpha, k). \tag{31}$$

The function f in (31) must be invertible, that is α and k can be computed uniquely from θ :

$$\begin{bmatrix} \alpha \\ k \end{bmatrix} = \Gamma^{-1}(\theta). \tag{32}$$

With definition (30) the process model (7) can be rewritten as follows:

$$\dot{\xi} = \Phi(\xi)\theta - D\xi + F_{in}, \tag{33}$$

where the matrix $\Phi(\xi)$ and the vector of the unknown parameters θ must be defined so that the equation (31) to be satisfied:

$$KG(\xi)\alpha = \begin{bmatrix} \alpha_1 SRX \\ \alpha_2 SX \\ -k_1 \alpha_1 SRX - k_2 \alpha_2 SX \\ -k_3 \alpha_1 SRX \end{bmatrix} = \Phi(\xi)\theta,$$

that is:

$$\Phi(\xi) = \begin{bmatrix} SRX & 0 & 0 & 0 & 0 \\ 0 & SX & 0 & 0 & 0 \\ 0 & 0 & -SRX & -SX & 0 \\ 0 & 0 & 0 & 0 & -SRX \end{bmatrix}, \tag{34a}$$

$$\theta = \begin{bmatrix} \alpha_1 \\ \alpha_2 \\ k_1 \alpha_1 \\ k_2 \alpha_2 \\ k_3 \alpha_1 \end{bmatrix}. \tag{34b}$$

The general form of an asymptotic parameter estimator has the form [2]:

$$\frac{d\hat{\xi}}{dt} = \Phi(\xi)\hat{\theta} - D\xi - \Omega(\xi - \hat{\xi}), \tag{35a}$$

$$\begin{aligned} \frac{d\hat{\theta}}{dt} &= \Phi^T(\xi)\Gamma(\xi - \hat{\xi}), \\ \begin{bmatrix} \hat{\alpha} \\ \hat{k} \end{bmatrix} &= \Gamma^{-1}(\hat{\theta}), \end{aligned} \quad (35b)$$

where: $\Omega \in R^{N \times N}$ is a diagonal matrix with negative elements $\omega_i (i=1, N)$; $\Gamma \in R^{N \times N}$ is diagonal matrix with positive elements $\gamma_i (i=1, N)$.

The parameter estimator for the class of processes under consideration is obtained in the form:

$$\begin{aligned} \frac{d\hat{X}}{dt} &= SRX\hat{\theta}_1 - DX + \omega_1(X - \hat{X}), \\ \frac{d\hat{A}}{dt} &= SX\hat{\theta}_2 - DA + \omega_2(A - \hat{A}), \end{aligned} \quad (36a)$$

$$\frac{d\hat{S}}{dt} = -SRX\hat{\theta}_3 - SX\hat{\theta}_4 - D(S - S_f) + \omega_3(S - \hat{S}),$$

$$\frac{d\hat{R}}{dt} = -SRX\hat{\theta}_5 - D(R - R_f) + \omega_4(R - \hat{R}),$$

$$\frac{d\hat{\theta}_1}{dt} = SRX\gamma_1(X - \hat{X}),$$

$$\frac{d\hat{\theta}_2}{dt} = SX\gamma_2(A - \hat{A}),$$

$$\frac{d\hat{\theta}_3}{dt} = -SRX\gamma_3(S - \hat{S}), \quad (36b)$$

$$\frac{d\hat{\theta}_4}{dt} = -SX\gamma_3(S - \hat{S}),$$

$$\frac{d\hat{\theta}_5}{dt} = -SRX\gamma_4(R - \hat{R}),$$

$$\hat{\alpha}_1 = \hat{\theta}_1,$$

$$\hat{\alpha}_2 = \hat{\theta}_2,$$

$$\hat{k}_1 = \frac{\hat{\theta}_3}{\hat{\alpha}_1} = \frac{\hat{\theta}_3}{\hat{\theta}_1}, \quad (36c)$$

$$\hat{k}_2 = \frac{\hat{\theta}_4}{\hat{\alpha}_2} = \frac{\hat{\theta}_4}{\hat{\theta}_2},$$

$$\hat{k}_3 = \frac{\hat{\theta}_5}{\hat{\alpha}_1} = \frac{\hat{\theta}_5}{\hat{\theta}_1}.$$

The specific rates can be calculated from:

$$\begin{aligned}
 \hat{V}_x &= \hat{S} \hat{R} \hat{\theta}_1, \\
 \hat{V}_a &= \hat{S} \hat{\theta}_2, \\
 \hat{V}_s &= \hat{S} \hat{R} \hat{\theta}_3 + \hat{S} \hat{\theta}_4, \\
 \hat{V}_r &= \hat{S} \hat{R} \hat{\theta}_5.
 \end{aligned} \tag{37}$$

The elements of the diagonal matrixes $\Gamma \in R^{4 \times 4}$ and $\Omega \in R^{4 \times 4}$ must satisfy the matrix equation derived from stability point of view [2]:

$$\Omega^2 = 4\Phi\Phi^T\Gamma, \tag{38}$$

that for the class of processes under consideration has the following form:

$$4\Phi\Phi^T\Gamma = \begin{bmatrix} 4\gamma_1(SRX)^2 & 0 & 0 & 0 \\ 0 & 4\gamma_2(SX)^2 & 0 & 0 \\ 0 & 0 & 4\gamma_3(SRX)^2 + 4\gamma_3(SX)^2 & 0 \\ 0 & 0 & 0 & \gamma_4(SRX)^2 \end{bmatrix}.$$

Simple equations for γ calculation are determined from the equation (38):

$$\begin{aligned}
 \gamma_1 &= \frac{\omega_1^2}{4(SRX)^2}, \\
 \gamma_2 &= \frac{\omega_2^2}{4(SX)^2}, \\
 \gamma_3 &= \frac{\omega_3^2}{4(SRX)^2 + 4(SX)^2}, \\
 \gamma_4 &= \frac{\omega_4^2}{4(SRX)^2},
 \end{aligned} \tag{39}$$

where ω_i ($i = 1, \dots, 4$) are the tuning parameters.

Case study: Lysine fermentation

Lysine production process is a typical example of a class of processes under consideration [4, 9, 11, 12, 17, 23-25]. As a case study industrial scale Lysine fermentation in a fed-batch culture of *Brevibacterium flavum* using the molasses' technology is considered [9]. The biochemical model, corresponding to the process and used for a real process simulation, has the form:

$$\frac{dX}{dt} = V_x X - \frac{F}{V} X, \tag{40a}$$

$$\frac{dA}{dt} = V_a X - \frac{F}{V} A, \tag{40b}$$

$$\frac{dS}{dt} = -V_s X + \frac{F}{V} (S_f - S), \tag{40c}$$

$$\frac{dR}{dt} = -V_r X + \frac{F}{V} (R_f - R), \quad (40d)$$

$$\frac{dC}{dt} = -V_c X + K_{La} (100 - C), \quad (40e)$$

$$\frac{dV}{dt} = F, \quad (40f)$$

$$V_x = a_{10} \frac{R}{(a_{11} + R)} \frac{C}{(a_{12} + C)}, \quad (41a)$$

$$V_a = a_{13} \frac{S}{(a_{14} + S)} \frac{a_{15}}{(a_{15} + S)} \frac{A}{(a_{16} + A)} \frac{C}{(a_{17} + C)}, \quad (41b)$$

$$V_s = (a_2 V_x + a_3 V_a + a_4), \quad (41c)$$

$$V_r = a_1 V_x, \quad (41d)$$

$$V_c = (a_5 V_x + a_6 V_a + a_7), \quad (41e)$$

$$K_{La} = a_8 + a_9 \frac{F_{air}}{V}, \quad (41f)$$

where: S_f is the sugar concentration in the feed, [g·l⁻¹]; R_f – growth factor concentration in the feed, [g·l⁻¹]; F_{air} – flow rate of the air, m³·h⁻¹; V_x – specific growth rate, [h⁻¹]; V_a – specific Lysine production rate, [h⁻¹]; K_{La} – volumetric mass-transfer coefficient, [h⁻¹]; a_i ($i = 1, \dots, 17$) – model parameters.

The model parameters, obtained on the basis of 143 industrial fermentations have the values given in Table 1.

Table 1. Biochemical model coefficients

a_1	a_2	a_3	a_4	a_5	a_6	a_7	a_8	a_9
0.068	1.15	2.62	$0.682 \cdot 10^{-3}$	$0.138 \cdot 10^4$	$0.421 \cdot 10^3$	60.7	-13.5	4.75
a_{10}	a_{11}	a_{12}	a_{13}	a_{14}	a_{15}	a_{16}	a_{17}	
0.81	1.2	54.8	0.357	17.4	172	23.6	47.9	

The typical initial conditions and the standard values of some other parameters for the case of fed-batch operation (suboptimal control of the process by the flow rate of feed supply F) are given in Table 2. It should be also noted that:

$$\begin{aligned} 0 < F < F^*, \quad V < V^*, \\ S(t_f) < S^*, \quad X, A, S, R, C > 0, \end{aligned} \quad (42)$$

where t_f is the end of the process – about 55-60 h for fed-batch mode of operation.

Table 2. Typical initial conditions and standard values of parameters

$X(0)$	$A(0)$	$S(0)$	$R(0)$	$C(0)$	$V(0)$
1.0	1.0 g·l ⁻¹	125 g·l ⁻¹	1.3 g·l ⁻¹	100%	56 m ³
V^*	F^*	S^*	S_f	R_f	F_a
75 m ³	1.3 m ³ ·h ⁻¹	4 g·l ⁻¹	220 g·l ⁻¹	0.95 g·l ⁻¹	5000 m ³ ·h ⁻¹

The following parameters of the suboptimal feed supply program have been as derived [9]: $t_1^0 = 23.5$ h; $t_2^0 = 40.8$ h; $F^0 = 1.3$ m³·h⁻¹. All the variables and specific rates are given in Fig. 1.

The General model of the Lysine production process, in this case has the form:

$$\begin{aligned}
 \frac{dX}{dt} &= \varphi_1 - DX = SRX\alpha_1 - DX, \\
 \frac{dA}{dt} &= \varphi_2 - DA = SX\alpha_2 - DA, \\
 \frac{dS}{dt} &= -k_1\varphi_1 - k_2\varphi_2 - D(S - S_f) = -SRXk_1\alpha_1 - SXk_2\alpha_2 - D(S - S_f), \\
 \frac{dR}{dt} &= -k_3\varphi_1 - D(R - R_f) = -SRXk_3\alpha_1 - D(R - R_f), \\
 \frac{dC}{dt} &= -k_4\varphi_1 - k_5\varphi_2 + Q_{in} = -k_4SRX\alpha_1 - k_5SX\alpha_2 + Q_{in}.
 \end{aligned} \tag{43}$$

The comparison between the biochemical model (40)-(41) with completely known kinetics, used for real-life process simulation, and the General model (43), with completely or partially unknown kinetics, used for estimation, leads to the following relations for the yield factors:

$$\begin{aligned}
 k_1 &= a_2 + \frac{a_4}{V_x} && \text{(valid for Phase A only),} \\
 k_2 &= a_3 + \frac{a_4}{V_a} && \text{(valid for Phase B only),} \\
 k_3 &= a_1 && \tag{44} \\
 k_4 &= a_5 + \frac{a_7}{V_x} && \text{(valid for Phase A only),} \\
 k_5 &= a_6 + \frac{a_7}{V_a} && \text{(valid for Phase B only).}
 \end{aligned}$$

The yield factors are functions not only of the model coefficients, but of the specific growth rates (V_x, V_a), too. It means that they are not constant and should be estimated as time-varying parameters. In case when they are accepted as known constant parameters (state estimator design), their values were estimated by optimization procedure (minimum least square error) fitting the main state variables generated from the two models (40)-(41) and (44).

The following main points should be especially noticed for the case of state estimator design:

Different yields factors participate in each of the two phases of the process – k_1, k_3, k_4 and k_2, k_3, k_5 for *Phase A* and *Phase B*, respectively.

1. Since it is accepted that $V_a \approx 0$ during the *Phase A*, and $V_x \approx 0$ during the *Phase B*, the second terms of the coefficients k_1, k_4 and k_2, k_5 can be omitted during the corresponding phases.
2. The range of variation of the different coefficients k_1, k_4 and k_2, k_5 can be estimated taking into account the range of variation of the specific rates V_x and V_a during *Phase A* and *Phase B* respectively.
3. The simulation results show significant fitting of the estimates and the model values of the variables, including in case of different initial conditions. A 5% white noise was added to the generated values of the measured variable C (Fig. 2).

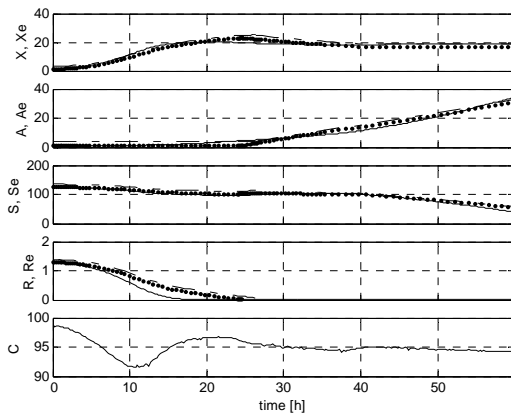


Fig. 2 State estimator: the model values X, A, S, R, C (solid line) and the estimated values X_e, A_e, S_e, R_e of the biomass, product, substrate and growth factor, under exact (dotted line) and inexact initial conditions (dashed line)

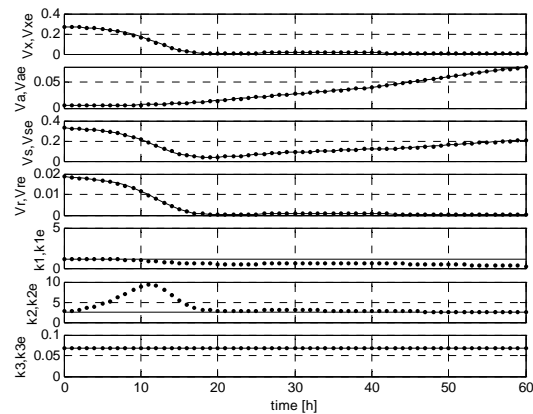


Fig. 3 Parameter estimator: the model values V_x, V_a, V_s, V_r (solid line) and the estimated values $V_{xe}, V_{ae}, V_{se}, V_{re}$ (dotted line) of the specific rates; the constant values k_1, k_2, k_3 (solid line) and the estimated values k_{1e}, k_{2e}, k_{3e} (dotted line)

In case of observer-based estimator design, i.e. the following main points should be noticed:

1. The parameter estimator was tuned by the values of $\omega_i, i = (1, 4)$ which was found to be:

$$\omega_1 = 10; \omega_2 = 38; \omega_3 = 23; \omega_4 = 10.$$

2. The simulation results show perfect fitting of the estimated and model values of the kinetic rates (Fig. 3).

As it could be expected, some difference exists between the estimated values and the constant values of the yield factors k_1 and k_2 (Fig. 3). The significant difference for k_1 and k_2 however appears during the *Phase B* and *Phase A* respectively, where the corresponding yield factor

does not participate in the Phase description (*Model B* and *Model A*). For example the estimated value of k_2 , the yield factor with respect to the Lysine production, is equal to the constant value during the production phase, but there exists big error during the growth phase.

Similar result is observed for the coefficient k_1 . However, in this case there is small difference during the second half of the growth phase too, where the product formation is negligible, but not completely zero.

3. Simulation investigations under different initial conditions for $\theta_i, i=1-5$ variables (36), which determine the estimated variables, are also done. The results show very good convergence properties of the estimator in case of wide range of different initial conditions for $\theta_i, i=1-5$. The specific rates and the yield coefficients, corresponding to changes in the initial conditions for $\theta_i, i=1-5$ variables (50% decrease of their exact initial values) are given in Fig. 4.

4. Simulation investigations under different initial conditions for the main process variables X, A, S, R are also done. The results show very good convergence properties of the estimator in case of wide range of different initial conditions. The specific rates and the yield coefficients, corresponding to the changes in the initial conditions for X, A, S, R variables (50% decrease of their exact initial values) are given in Fig. 5.

The results confirm the applicability of the designed software sensors. It should be especially noticed, that in general, the two estimators could be used for continuous fermentation too. However in this mode of operation, where the steady state is the main regime, the dissolved oxygen is usually kept constant and it could not be used as main on-line measurable variable for the case of state estimator design.

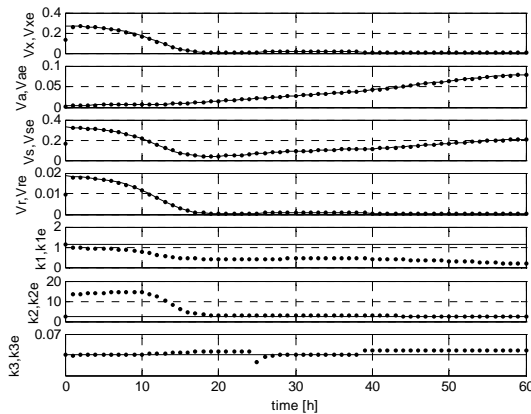


Fig. 4 For $\theta_i, i=1-5$

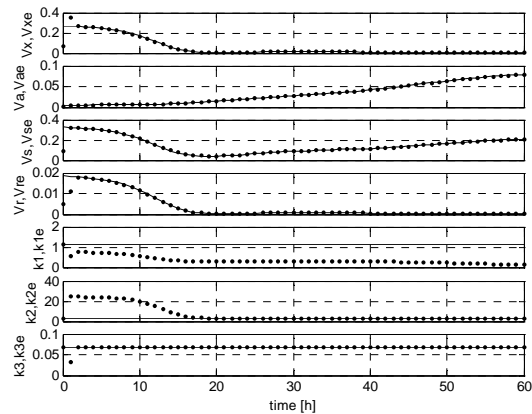


Fig. 5 For X, A, S, R

(50% decrease of the exact initial conditions) (50% decrease of the exact initial conditions)
 Parameter estimator: the model values V_x, V_a, V_s, V_r (solid line) and the estimated values $V_{xe}, V_{ae}, V_{se}, V_{re}$ (dotted line) of the specific rates; the constant values k_1, k_2, k_3 used for state estimator design (solid line) and the estimated values k_{1e}, k_{2e}, k_{3e} (dotted line) of the yield coefficients under inexact initial conditions.

Conclusion

Present paper is aimed to give solution of the measurement problems in bioreactors with respect to the biological variables, on the basis of only one relatively easy measurable variable, thus creating a firm basis for adaptive control systems design. Two separate sensors (state and parameter estimators) for a class of aerobic fermentation processes are designed. The state estimator is developed based on knowledge of *only one* on-line measurable variable, the dissolved oxygen, and the yield factors assumed as constant coefficients. Parameter estimator of the specific reaction rates is developed under the assumption that all the process variables are known on-line by measurements or estimates. The yield factors are also estimated as non-stationary parameters. Thus a basis for comparison with the specified constant values used for the state estimator design is created. As a case study industrial Lysine fermentation in fed-batch mode of operation is considered. Simulation results under different operating conditions are given in order to highlight the performances of the proposed sensors. The sensors can be used for (easily adapted to) any bioprocess that belongs to the defined class (for example bio-ethanol production). Further research is going on to estimate the software sensors performance in an adaptive control system.

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