Dynamics Monitoring of Fed-batch *E. coli* Fermentation

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Abstract: *A new method for on-line dynamics monitoring of three physiological states of fed-batch E. coli fermentation is proposed. The method is based on the acetate kinetics accepted as adaptive key parameter for recognition of current physiological state. Software sensors of main kinetic parameters are derived. They are connected in cascade scheme with inputs on-line measurements of acetate and glucose concentrations. In the outputs the on-line information of three biomass growth rates and biomass concentration is received. Tuning of the estimation algorithms is realized. The efficiency of the proposed method is investigated by simulations using a new biochemical process model. Discussion about the accuracy of obtained estimates and their relationship with the values of tuning parameters is done. The proposed method could be applied for control algorithms design for each physiological state.*

Keywords: *Software sensor, Fed-batch fermentation, Oxidative and fermentative states, E. coli.*

Introduction

The successful optimization of bioprocesses is infeasible without accurate monitoring all states in a microbial cell culture that are critical for product yield and productivity. One of the most effective ways of achieving this is by real-time data analysis as acquired from sensors capable of measuring the relevant process parameters [12]. Such observations can be included in automated and supervised feedback control to support maintaining favorable conditions for good product quality and quantity [22]. Unfortunately, the access to sensors which meet the requirements for process on-line monitoring is limited.

A solution of this problem is to utilize the information from hardware sensors as inputs to more or less sophisticated mathematical models, which are able to generate the values of critical variables and parameters from the modeled biological phenomena. The combination of hardware sensors with estimation algorithms implemented to software used to provide on-line estimations of unmeasurable variables and parameters is known as a software sensor (SS) [5].

Till now, several principal classes of software sensors are described in the literature for on-line monitoring of biotechnological processes. One class includes the mechanistic models derived from first principles, usually using basic mass balance and kinetic equations [16, 18, 24]. Another class is presented by the classical observers, which are based on the perfect knowledge of both model structure and parameters, such as the Luenberger and the Kalman observers [1, 8]. In spite of the satisfactory results reported, an uncertainty in the model

parameters can generate a large bias in the estimates. Third class comprises asymptotical observers (AO) [2, 13, 23], observer-based estimators [2, 7, 10], some groups of nonlinear [3, 4, 6, 19] and adaptive observers [2, 9, 10]. All of these methods are independent in different degree from the process kinetics knowledge. A potential problem related to asymptotical observers is the dependence of the estimation convergence rate on the operational conditions [2]. The software sensors from this class has the advantage to give an acceptable decision of ones of the main challenges in bioprocess monitoring – the lack of process reproducibility and uncertainty of parameter's values. An alternative to these mechanistic approaches are software sensors based on "black-box" models where inputs are correlated to outputs via a non-deterministic relationships [20]. Although the considered above software sensors refer to different bioprocesses, only few examples concern their implementation to complex ones, described by dynamical models containing several balance equations with complex kinetics.

Such process is high-cell density fed-batch fermentation of *E. coli* that is characterized by different physiological states [16-18] during the cultivation and respectively by multiple growth rates of biomass, which is directly related to the target product – proteins. According to the approach in [2], the reaction scheme of such process could be presented as set of the following three main reactions (metabolic pathways), which correspond to the process physiological states:

- Oxidative growth on glucose, with specific growth rate μ_1:

$$k_1 S + k_5 O \xrightarrow{\mu_1} X + k_8 C \quad (1)$$

- Fermentative growth on glucose, with specific growth rate μ_2:

$$k_2 S + k_6 O \xrightarrow{\mu_2} X + k_3 C + k_9 A \quad (2)$$

- Oxidative growth on acetate, with specific growth rate μ_3:

$$k_4 A + k_7 O \xrightarrow{\mu_3} X + k_{10} C \quad (3)$$

where X, S, A, O and C respectively concern rations of biomass, glucose, acetate, dissolved oxygen and carbon dioxide in the culture broth, k_1-k_{10} – yield coefficients.

In the literature, several adaptive approaches for on-line estimation of partially known process kinetics and/or unmeasured state variables of this process are proposed [9, 11, 23]. In [23], a comparison of asymptotical observers and Extended Kalman Observer is done as estimating unmeasurable state variables of the process using on-line measurements of dissolved oxygen and carbon dioxide concentrations. The obtained results showed that the performance of AO in the fermentation monitoring was slightly better. In [9], the proposed approach for on-line monitoring of three biomass growth rates and biomass concentration is based on adaptive observer theory, on-line measurements of dissolved oxygen and carbon dioxide concentrations as well as laboratory measurements of biomass concentration. In spite of adaptive properties of the proposed software sensors which estimate the biomass growth rates as unknown time-varying parameters, the large number of tuning parameters is not favorable the used-friendly synthesis of the cascade estimation scheme. In contrast to [9], the derived in [11] adaptive software sensors of the same growth rates are based on two sub-models describing oxidative-fermentative growth on glucose and oxidative one on glucose and acetate. The key parameter for states recognition is the critical value of glucose consumption rate, accepted as constant. But in practice this value is time-varying one from one experiment to the other ones due to its dependence from type of strain and lack of reproducibility of the bioprocesses.

In this paper, a new method for on-line monitoring of dynamics of physiological states typical for fed-batch *E. coli* fermentation is proposed. The method is based on an adaptive key parameter allowing on-line recognition of current physiological state. For realization of the new method, a four-step cascade scheme of software sensors is developed. Inputs of the scheme are on-line measurements of acetate and glucose concentrations. In first step, acetate production or consumption rate is estimated in dependence from acetate measurements. The output of the scheme includes the growth rates of biomass and its concentration. The properties of the estimation algorithms are investigated by simulations under different values of tuning parameters using the proposed in [26] biochemical model of the process. Conclusions about the applicability of the monitoring scheme are done.

Development of cascade scheme for process dynamics monitoring

Operational model of the process

The derivation of software sensors' scheme requires a development of operational model corresponding to the three reactions from the scheme (1)-(3). According [2, 11, 15], it consists of two sub-models (4) and (5) describing the oxidative-fermentative growth on glucose and oxidative growth on glucose and acetate, respectively as follows:

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ A \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ -k_1 & -k_2 \\ 0 & k_3 \end{bmatrix} \begin{bmatrix} \mu_1(t) \\ \mu_2(t) \end{bmatrix} X - \frac{F_{in,s}}{W} \begin{bmatrix} X \\ S \\ A \end{bmatrix} + \frac{F_{in,s}}{W} \begin{bmatrix} 0 \\ S_{in} \\ 0 \end{bmatrix}, \quad (4)$$

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ A \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ -k_1 & 0 \\ 0 & -k_4 \end{bmatrix} \begin{bmatrix} \mu_1(t) \\ \mu_3(t) \end{bmatrix} X - \frac{F_{in,s}}{W} \begin{bmatrix} X \\ S \\ A \end{bmatrix} + \frac{F_{in,s}}{W} \begin{bmatrix} 0 \\ S_{in} \\ 0 \end{bmatrix}, \quad (5)$$

where $F_{in,s}$ is the feed rate, S_{in} – glucose concentration in the feed solution, W – weight of reactor.

The growth rates $R_{X1} = \mu_1 X$, $R_{X2} = \mu_2 X$ and $R_{X3} = \mu_3 X$ could be considered as unknown time-varying parameters that have to be estimated using available on-line information.

Cascade software sensor – structure derivation and stability analysis

The general scheme is presents in Fig. 1. The software sensors are derived under the following assumptions:

- ✓ The models (4), (5) parameters related to transport dynamics – feed rate, glucose concentration in the feed solution and the weight of reactor are known;
- ✓ The yield coefficients k_1-k_4 are known constants;
- ✓ On-line measurements of acetate A and glucose S concentrations are available. As it is stated in the literature, these two concentrations can be measured on-line by near infrared spectroscopy, high-performance liquid chromatography (HPLC) [21, 25] as well as flow injection analysis (FIA) [14].

In the case when time-derivative of acetate measurements, $dA/dt > 0$, this is an indication that the microorganisms are in oxidative-fermentative growth. The monitoring of this physiological state includes SS of acetate production rate, R_{ap}, that is input for the SS of fermentative growth rate, R_{X2}, which is input for SS of oxidative growth rate, R_{X1}. The received estimates of R_{X1} and R_{X2} provide the possibility on-line estimates of biomass concentration to be calculated. If $dA/dt < 0$, the microorganisms grow oxidatively on acetate and glucose. The monitoring of this physiological state includes SS of acetate consumption

rate that is input for the SS of oxidative growth rate on acetate, R_{X3}. Analogically to the oxidative-fermentative state, on the basis of estimates, R_{X1} and R_{X3}, these ones of X are received. The case $dA/dt = 0$ can be observed at the process beginning when there is no acetate in the culture broth and microorganisms grow on glucose only. Zero value of this time-derivative occurs also when the accumulated acetate in the culture broth begins to consume.

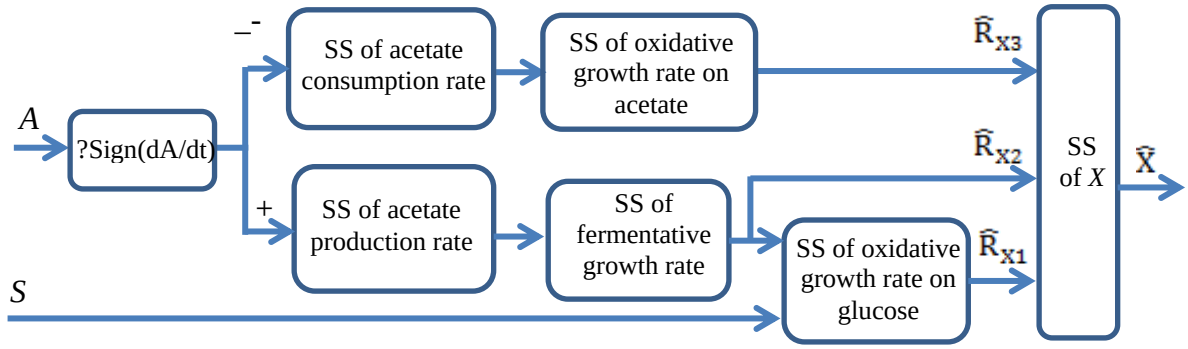

Fig. 1 Software sensor' scheme

On-line estimation of acetate production rate

The software sensor of acetate production rate, R_{ap}, is activated when the time-derivative of acetate measurements, $dA/dt \geq 0$. The input is on-line measurements of acetate concentration. According to the approach proposed in [7], the estimator structure is presented by the following system:

$$\frac{d\hat{A}}{dt} = \hat{R}_{ap} - \frac{F_{in,s}}{W} A + w_1 (A - \hat{A}), \tag{6a}$$

$$\frac{d\hat{R}_{ap}}{dt} = w_2 (A - \hat{A}), \tag{6b}$$

where $\hat{R}_{ap}$ and $\hat{A}$ are the estimates of acetate production rate, R_{ap}, and acetate concentration, respectively, A – the measured values, w_1 and w_2 – estimator' tuning parameters, which values have to satisfy stability conditions.

Stability analysis

Defining the errors $\tilde{A} = A - \hat{A}$ and $\tilde{R}_{ap} = R_{ap} - \hat{R}_{ap}$, the following system is derived from (6):

$$\frac{d\mathbf{x}}{dt} = \mathbf{A}\mathbf{x} + \mathbf{v}, \tag{7}$$

with $\mathbf{x} = \begin{bmatrix} \tilde{A} \\ \tilde{R}_{ap} \end{bmatrix}$, $\mathbf{A} = \begin{bmatrix} -w_1 & 1 \\ -w_2 & 0 \end{bmatrix}$, $\mathbf{v} = \begin{bmatrix} 0 \\ \dfrac{dR_{ap}}{dt} \end{bmatrix}$.

Let h_1 and h_2 are real eigenvalues of matrix **A** related by definition to w_1 and w_2 as follows:

$$w_1 = -(h_1 + h_2) \text{ and } w_2 = h_1 h_2. \tag{8a}$$

The real values avoid induced oscillations in the estimates that do not correspond to any physical phenomenon related to the estimated reaction rates.

If additionally the eigenvalues are equal $h_1 = h_2 = h$ with h – negative constant to be satisfied stability of the system (6), then the relationships (8a) are rewritten:

$$w_1 = -2h,\ w_2 = w_1^2 / 4. \quad (8b)$$

The selection of a double eigenvalue has several advantages:

(i) the degrees of freedom of the algorithm are reduced,
(ii) it allows an easy interpretation in terms of convergence, and
(iii) the calculation of the tuning parameters is straightforward.

Applying the relationships (8b), the tuning of the estimation algorithm is reduced to the choice of one design parameter h. As will be shown in the section "results and discussions", the value of this parameter will be investigated depending on requirements for estimate's convergence rate and their sensibility with respect to the disturbance included in the vector **v**.

On the basis of the estimates, $\hat{R}_{ap}$, the following on-line information could be received using the following three steps:

On-line estimation of fermentative growth rate

The estimates of the fermentative biomass growth rate, $\hat{R}_{X2}$, are obtained using the relationship between R_{X2} and acetate production rate, R_{ap}, as follows:

$$\hat{R}_{X2} = \hat{R}_{ap} / k_3, \quad (9)$$

where k_3 is the yield coefficient as presented in the model (4).

On-line estimation of oxidative growth rate on glucose

The next step of the scheme from Fig. 1 includes the following software sensor of R_{X1}:

$$\frac{d\hat{S}}{dt} = -k_1\hat{R}_{X1} - k_2\hat{R}_{X2} - \frac{F_{in,s}}{W}S + \frac{F_{in,s}}{W}S_{in} + w_3(S - \hat{S}), \quad (10a)$$

$$\frac{d\hat{R}_{X1}}{dt} = w_4(S - \hat{S}), \quad (10b)$$

where $\hat{R}_{X2}$ are the estimates of R_{X2} from (9), $\hat{R}_{X1}$ – the estimates of R_{X1}, w_3 and w_4 – estimator (10) parameters which values are chosen according the procedure proposed above based on stability analysis and choice of a tuning parameter. For SS (10) the relationships between w_3, w_4 and h are as follows:

$$w_3 = -2h,\ w_4 = -w_3^2 / 4k_1. \quad (11)$$

The growth rate, R_{X1}, exists always when the process is in fed-batch mode, i.e. there is glucose feeding. The growth rate, R_{X2}, appears when specific glucose consumption rate is equal or higher than so called critical one as mentioned in [11] and given below in details in Eq. (16). In opposite case, $\hat{R}_{X2} = 0$.

The on-line monitoring of the process dynamics at oxidative-fermentative state could be realized by using Eqs. (6), (8b) and (9-11).

When the time-derivative of acetate measurements, $dA/dt \leq 0$ and $A \neq 0$, that is sign for beginning of the acetate consumption and a SS of acetate consumption rate is derived using acetate measurements.

On-line estimation of acetate consumption rate

The structure of the proposed SS is similar to this one described by the system (6):

$$\frac{d\hat{A}}{dt} = \hat{R}_{ac} - \frac{F_{in,s}}{W} A + w_5 (A - \hat{A}), \quad (12a)$$

$$\frac{d\hat{R}_{ac}}{dt} = w_6 (A - \hat{A}), \quad (12b)$$

where $\hat{R}_{ac}$ and $\hat{A}$ are the estimates of acetate consumption rate, R_{ac}, and acetate concentration, A, respectively, w_5 and w_6 – estimator' tuning parameters. Since the values of $\hat{R}_{ac}$ are negative ones, the tuning parameters are calculated by the following expressions:

$$w_5 = -2h, \; w_6 = -w_5^{\,2} / 4 \quad (13)$$

and the tuning consists of choice of one parameter – the eigenvalue h.

On the basis of the estimates $\hat{R}_{ac}$ the following on-line information could be received:

On-line estimation of oxidative growth rate on acetate

On-line information for the oxidative biomass growth rate on acetate, R_{X3}, is obtained using the relationship between $\hat{R}_{ac}$ and R_{X3} as follows:

$$\hat{R}_{X3} = -\hat{R}_{ac} / k_4, \quad (14)$$

where k_4 is the yield coefficient as presented in the model (5), $\hat{R}_{X3}$ – estimates of R_{X3}.

The on-line monitoring of the process dynamics at oxidative growth on glucose and acetate could be realized by using Eqs. (10), (12)-(14).

On-line estimation of biomass concentration

It is evident that if all physiological states, presented by the scheme (1)-(3), occur during the process, the estimation of biomass concentration has to be realized using the sum of the estimates of three growth rates as follows:

$$\frac{d\hat{X}}{dt} = \hat{R}_{X1} + \hat{R}_{X2} + \hat{R}_{X3} - \frac{F_{in,s}}{W} \hat{X}. \quad (15)$$

Depending on the current physiological state, different combinations of these growth rates determine the biomass growth kinetics.

Results and discussion

Simulation investigations of the proposed estimation scheme are realized using the recently proposed in [26] unstructured process model based on the operational models (4), (5). The oxidative-fermentative growth on glucose is presented by sub-model (4) with the following kinetic equations for specific growth rates μ_1 and μ_2:

$$\mu_1 = q_{s,crit} / k_1, \quad (16a)$$

$$\mu_2 = (q_s - q_{s,crit}) / k_2, \quad (16b)$$

with $q_s = \frac{q_{s,\max} S}{K_S + S}$ and $q_{s,crit} = \frac{q_{o,\max}}{k_{os}} \frac{K_{i,o}}{K_{i,o} + A}$.

According to the model (4), the acetate production rate is presented by:

$$R_{ap} = k_3 \mu_2 X \,. \tag{16c}$$

The oxidative growth on glucose and acetate is presented by the sub-model (5) with the following kinetic equations for specific growth rates μ_1 and μ_3:

$$\mu_1 = q_s / k_1 \tag{17a}$$

$$\mu_3 = q_{ac} / k_4 \tag{17b}$$

with $q_{ac} = \dfrac{q_{ac,\max}}{(K_a + A)} \dfrac{AK_{ia}}{(K_{ia} + A)}$ – specific rate of acetate consumption.

According to the model (5), the acetate consumption rate is presented by:

$$R_{ac} = -k_4 \mu_2 X \,. \tag{17c}$$

The proposed adaptive marker for state recognition and for switching the sub-models describing the different physiological states is based on on-line information for acetate concentration and transport dynamics as follows:

$$R_{ac} = \frac{dA}{dt} + \frac{F_{in,s}}{W} A. \tag{18}$$

For the purposes of parametric identification of the proposed model, realized in [26], applied program packages in MATLAB environment are developed using genetic algorithm. The experimental data from fed-batch fermentation with *E. coli* carried out in fermentation laboratory in University of Minho, Portugal were applied [15]. The optimization criterion is minimal total relative mean square error between experimental data of biomass, glucose and acetate concentrations and corresponding model data. The parametric identification' results using different models for specific rates of glucose consumption, q_s, and acetate consumption, q_{ac}, as well the errors on optimization criterion are given in [26]. In Table 1, the identification result using the best structure of the model – kinetic equations (16), (17) is given.

Table 1. Kinetic parameter's values

Parameter	$q_{s,max}$	K_S	k_1	k_2	k_3	k_4	k_{os}	$q_{o,max}$	$K_{i,o}$	$q_{ac,max}$	K_a	K_{ia}
Values	11.34	7.17	2.06	3.17	0.72	8.9	5.47	0.28	2.8	0.04	0.37	84.8

In all figures, on-line measurements of acetate and glucose concentrations simulated by the model described by Eqs. (4), (5), (16), (17) and (18) are presented with continuous line and the experimental data with circles. In Figs. 2 and 3, the investigation results of oxidative-fermentative physiological state are presented. This state exists when the time-derivative, *dA/dt*, accepts positive values only.

In Fig. 2, the results for acetate production rate obtained by software sensor (6) are presented. The model data of this parameter are compared to its estimates in sub-figure 2b. Two eigenvalues are tested: $h = -25$ and $h = -50$. Their choice is a compromise between the estimates convergence and their sensibility with respect to the disturbance dR_{ap} / dt. The results are with slightingly small differences in the estimates of acetate production rate and acetate concentration for the two cases as can be seen in sub-figures 2b and 2a respectively. The estimate's errors of R_{ap}, presented in sub-figure 2c, demonstrate better convergence and accuracy at higher eigenvalue especially in the moments of more

considerable changes of time-derivative dR_{ap}/dt which act as disturbance according the system error (7). The maximal relative error reaches 14% for h = –25 and 5% for h = –50.

The simulation results of oxidative growth, R_{X1}, received by SS (10) are shown in Fig. 3. The observed in sub-figure 3a difference between the model and experimental data of glucose concentration is due to the inaccuracy of laboratory measurements at low values of glucose as is explained in [15]. In sub-figure 3b, a comparison between the model and estimation data of R_{X1} is presented. Since one of inputs of the SS (10) is proportional to $\hat{R}_{ap}$ according (9), the errors shown in sub-figure 2c appear as errors between 5 and 10 h in sub-figure 3c. The errors at the beginning and at the end of the state are due to the disturbance dR_{X1}/dt.

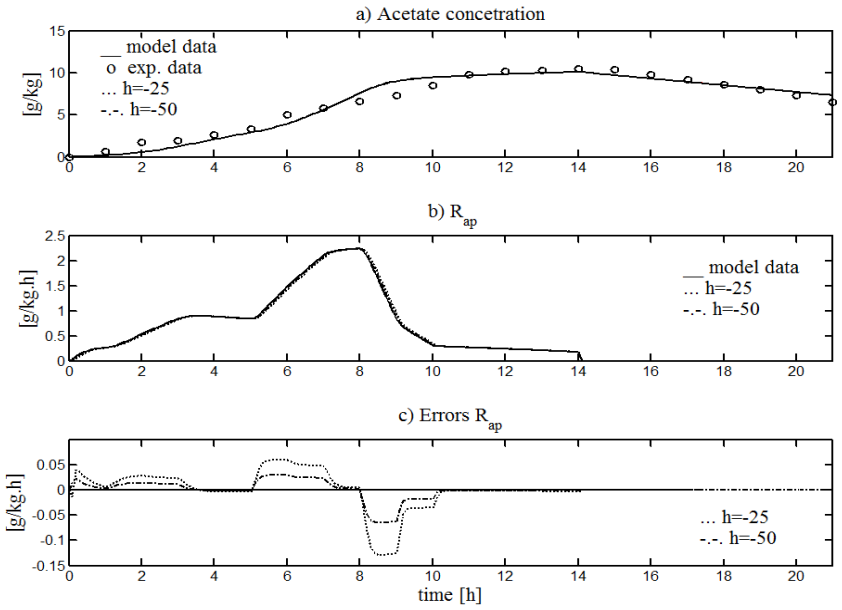

Fig. 2 Estimation of acetate production rate

After 14 h of fermentation, the glucose feed stops according the experiment carried out in [15] and hence glucose in the culture broth and the rate of oxidative growth on this substrate quickly go to zero. Hence the biomass will grow on acetate only.

In Fig. 4, the investigation results related to oxidative growth on acetate are presented. This state exists when the time-derivative, dA/dt, accepts negative values only. In sub-figure 4b the estimates of acetate consumption rate, R_{ac}, obtained by SS (12) using the same values of tuning parameter (with points and dashed line), are compared to the model data (with continuous line). The profile of this rate is stepwise one with start time that coincides with the beginning of acetate decreasing in the culture broth as can be seen on sub-figure 4a. The low constant values of the estimates are due to the low acetate consumption. The obtained estimates using two eigenvalues appear with a delay (in the order of minutes) that is smaller for the higher value (h = –50). Due to this delay the errors are highest at the estimation beginning after that quickly converge to zero. The estimates of oxidative growth rate on acetate, R_{X3}, are received using the Eq. (14).

As was mentioned above, the target product of this process is the protein, which is related to the biomass production. So, the estimates of biomass concentration, shown in Fig. 5, are very

valuable information for process optimization. In sub-figure 5a, the model values are compared to the estimates. They are obtained using the proposed above expression (15).

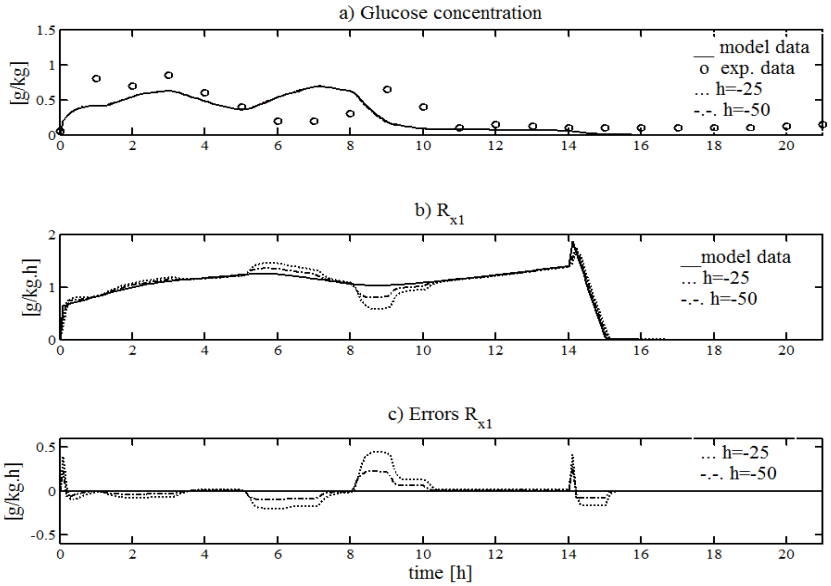

Fig. 3 Estimation of oxidative growth on glucose

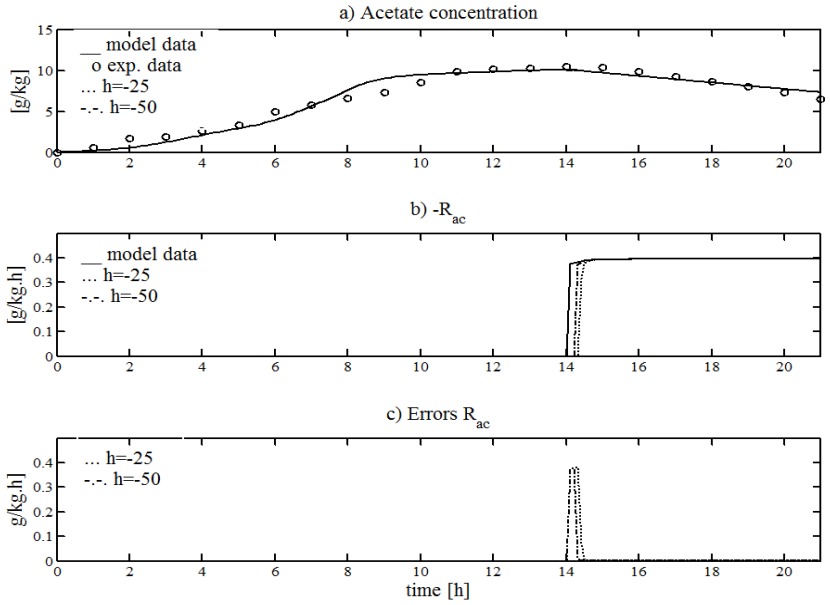

Fig. 4 Estimation of acetate consumption rate

As can be seen, the estimates and the model data have very close values. The profiles of estimation errors for the two eigenvalues plotted in sub-figure 5b have similar dynamics but smaller errors using higher eigenvalue. The maximal relative error is around 2% for h = –50 and 4% for h = –25 that is very good result with respect to estimation accuracy. A small bias in the estimates after 15 h of fermentation is observed. This can be explained with the lack of feeding that leads to maintenance of the initial deviation in biomass estimates till the end of fermentation.

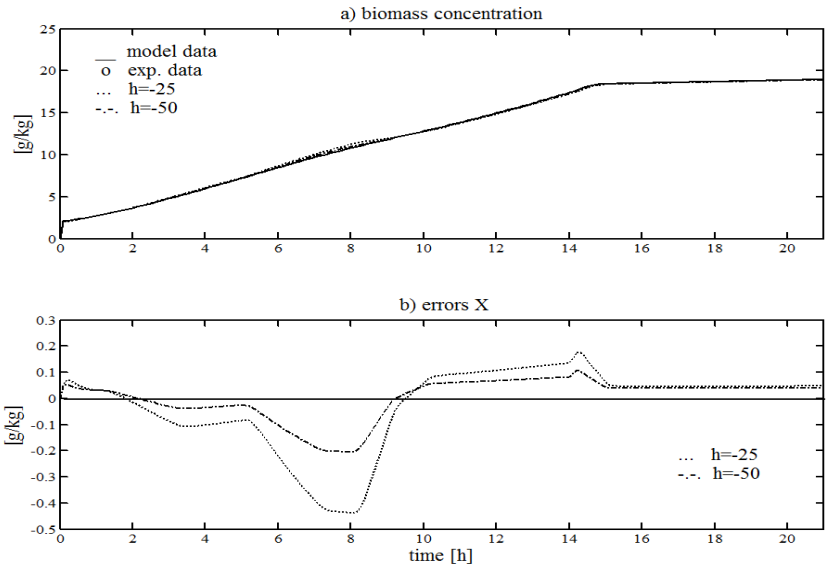

Fig. 5 Estimation of biomass concentration

On the basis of the estimates of biomass concentration and these ones of the three biomass growth rates, the corresponding specific growth rates could be calculated using the well-known relationship.

Conclusion

The proposed new method for on-line dynamics monitoring of fed-batch *E. coli* fermentation is based on the estimation of the acetate kinetics as key parameter for recognition of physiological state' change. On-line information of this parameter allows a cascade scheme of software sensors to be developed which outputs are the main process parameters and unmeasured biomass concentration. A user-friendly tuning of the proposed estimation algorithms is investigated. The simulation results demonstrate the good quality of the monitoring using different values of the tuning parameters. The received estimates of three growth rates of biomass and its concentration hold out the prospect the proposed method to be used for control algorithms design depending on the target product.

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