MONITORING THE INDUSTRIAL AEROBIC FERMENTATION PROCESS IN REAL TIME

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Abstract

Based on measurements of either oxygen concentration in exhaust gas or reaction heat flow, the specific growth rate and biomass concentration during the fermentation of the bacitracin in the 80 m^3 industrial batch bioreactor were determined in real time. Used one-parametric linear recursive least squares model with time-varying forgetting factor follows well the non-linear and, in certain phases of the production, very dynamic process of fermentation, and enables simulation of disturbances in industrial process. Estimated parameters agree well with the measurements, acquired off-line, and represent useful new information, which enable better monitoring of the industrial process.

INTRODUCTION

The bacitracin fermentation process is characterized by the complex dynamics and highly interlinked sub-processes of the biological transformations. Reliable monitoring and control of the industrial production of the bacitracin requires quality information on the process in real time, which can significant upgrade the production capability. There are many measurement techniques, which enable gathering the directly or indirectly measured physical, chemical and biological parameters of the process. However, due to limiting factors, such as reliability, risk of medium contamination, difficulties in transferring the results from pilot plants to industrial fermentors and complexity of the process, the real-time optimisation of the industrial fermentation process is not an easy task[1,2].

Various approaches can be used to determine unknown and unmeasurable parameters and state of the process. However, the design of stable estimator for bioprocess remains a complex task that must be studied for each particular process[3]. In

the studied case, an approach with separate parameter and state determination was chosen, where experimental modelling is used to determine specific growth rate and biomass concentration on-line and in real time during the industrial bacitracin fermentation in an 80 m^3 batch bioreactor.

Production of the bacitracin as a secondary metabolite is an aerobic process. For such processes, Bastin and Dochain[4] suggested the use of an asymptotic observer, which enables process state estimation based on partial measurement of state variables. Cazzador and Lubenova[5] proposed a nonlinear observer on the basis of the balance equation for *OUR* (oxygen uptake rate), which takes into account the dynamics of specific growth rate. Estler[6] suggested separate estimations of the process parameters and of the process state, which enabled estimation also of fast changeable process parameters. Charbonnier and Cheruy[3] used a model based only on the measurement of the partial pressure of released carbon dioxide in the fermentor's exhaust gas.

For real time monitoring of the specific growth rate and biomass concentration during the bacitracin fermentation in an 80 m^3 industrial batch bioreactor the estimator was developed, based on least square model, which was upgraded with time-varying forgetting factor to enable reliable estimation also during phases of fast and large changes in the process. The measured input signals of the estimator were either the reaction heat flow or the oxygen uptake rate.

MATERIALS AND METHODS

Process parameters measurement

On-line measurements of oxygen and carbon dioxide concentrations in the fermentor's exhaust gas and reaction heat flow were conducted during bacitracin fermentation in an industrial 80 m^3 batch reactor. The concentration of oxygen in the fermentor's exhaust gas was measured using an IJS MK 100 gauge operating on the principle of oxygen diffusion between actual and reference states.

In order to determine the reaction heat flow the following process parameters were measured on-line: the inlet and the outlet temperature of the cooling water; the inlet and

the outlet air temperature; the temperature of the broth in the fermentor using sterilisable Pt-100 probes; the flow of cooling water through the calibrated valve; the flow of air using a Heinrichs Messtechnik TSK-200 meter; the humidity of the inlet and the outlet air using a Vaisala HMP 234 meter; and the power of the mixer using the electric power consumed by the driving electro-motor and the known efficiency of the transmission and the rotor.

The biomass concentration in the broth c_X was measured off-line with the 'spread plate count' method[7] in three parallel runs.

Estimation of the specific growth rate based on oxygen uptake rate

Oxygen in the exhaust gas

Aerobic batch process can be described with the dynamic model[4]:

$$\frac{d}{d\tau}\begin{bmatrix} c_X \\ c_{O2} \end{bmatrix} = \begin{bmatrix} 1 \\ k_C \end{bmatrix} c_X \mu - \begin{bmatrix} 0 \\ Q_{O_2 in} - Q_{O_2 out} \end{bmatrix}, \tag{1}$$

where $Q_{O_2} = \frac{\dot{m}_{O_2}}{V_b}$ is the oxygen mass ratio with regard to the liquid volume in the fermentor.

The oxygen uptake rate *OUR* can be defined as[8]

$$OUR = (Y_{O_2/X}\mu + k_{mainO_2})c_X \,. \tag{2}$$

In the given case, the biomass concentration c_X can not be measured on-line and is therefore determined off-line with the time consuming 'spread plate count' method. The *OUR* can be determined on-line by measuring the oxygen concentration in the exhaust gas. The specific growth rate, μ, is an unknown and time-varying parameter, while the oxygen yield coefficient, $Y_{O_2/X}$ and the oxygen maintenance rate k_{main,O_2}, do not have to be known since in the model they appear as a ratio. For this reason and because of the short-interval sampling, it can be assumed that they are in a quasi-stationary state.

From equations (1) and (2) we can obtain a balance equation for *OUR*, which with the help of Euler's first order discretisation can be rearranged as:

$$\frac{OUR(n+1)-OUR(n)}{\Delta\tau}=\mu(n)OUR(n)\left[1-\frac{\frac{\mu(n+1)}{\mu(n)}-1}{\Delta\tau\left[\mu(n)+\frac{k_{main,O2}}{Y_{O_2/X}}\right]}\right]. \quad (3)$$

Estler[6] proposed a simple model for the estimation of specific growth rate on the basis of a measurement of the partial pressure of oxygen in the exhaust gas of the fermentor. In his model he assumes that μ varies very slowly and that its dynamics are negligible in the *OUR* balance equation, expressed in the parenthesis of the equation (3). This hypothesis might prove to be problematic, because it can result in biased estimations of μ when the latter is subject to significant variations.

To achieve both reliability and unbiased estimation we have selected a linear model, in which, similarly to Estler[6], the dynamic part in brackets was omitted in Eq. (3)

$$\frac{OUR(n+1)-OUR(n)}{\Delta\tau}=\mu(n)OUR(n), \quad (4)$$

and nonlinear dynamics of $\mu(n)$ was included in the model with a mechanism of weighting of a recursive algorithm with a variable forgetting factor $\lambda(n)$. This enables the model to track the process also during large changes, when linearisation is no longer satisfactory. The analysis of the adequacy of the proposed model is shown in Golobič et al.[9].

The specific growth rate can be expressed as

$$\mu(n)=\frac{\hat{a}(n)-1}{\Delta\tau}, \quad (5)$$

where $\hat{a}(n)$ is a parameter estimated with equation

$$OUR(n+1)=\hat{a}(n)OUR(n) \quad (6)$$

using the recursive least-squares method[10] with a variable forgetting factor[11].

In Estler[6] was shown that, in calculating the oxygen uptake rate, *OUR*, the variation of oxygen concentration in the broth, $dc_{O_2}/d\tau$, is negligible in comparison with the variation of oxygen transfer rate, and that, further, the oxygen concentration in

the broth is in a quasi-stationary state. With these assumptions, *OUR* can be calculated on the basis of oxygen concentration measurements in the exhaust gas, y_{02out}, as

$$OUR = \frac{M_{O_2}}{V_b M_{air} 100}\left[\dot{m}_{air,in} y_{O_2 in} - \dot{m}_{air,out} y_{O_2 out} \right] . \quad (7)$$

Variation of *OUR* during the industrial fermentation of bacitracin is shown in figure 2.

Estimation of the specific growth rate based on reaction heat flow

The reaction heat flow, released during the fermentation process, is the result of the biological activity of microorganisms. It is defined by an energy balance in the fermentor. The amount of reaction thermal energy, which is generated within a control volume, must equal the amount of thermal energy, which leaves the control volume, minus the amount of mechanical energy, which enters the control volume, minus the increase in the amount of energy stored in the control volume. The reaction heat flow can be expressed as:

$$q_r = q_w + q_{air} + q_{loss} - P_m - (m_b c_{p,b} + m_{wall} c_{p,wall}) \frac{\Delta T_b}{\Delta \tau} . \quad (8)$$

where q_w is the heat removed by the cooling water, q_{air} is the heat removed by air and q_{loss} are the heat losses through the walls of the reactor. P_m represents the heat flow resulting from the action of the mixer. $m_b c_{p,b}$ and $m_{wall} c_{p,wall}$ are the heat capacities of the broth and the walls of the fermentor, respectively. ΔT_b represents the change in the temperature of the broth in the specified time interval $\Delta \tau$.

Adequate measurement of the q_r requires j measurements of the parameters with the short sample time $\Delta \tau_j$ and the appropriate averaging of the signals over the observed interval $\Delta \tau$,

$$\Delta \tau = \sum_j \Delta \tau_j . \quad (9)$$

In the observed time interval, the heat removed by the cooling water can be expressed as

$$q_w = \left\{ \sum_j \dot{m}_{w,j} c_{p,w} \left(T_{w,j,out} - T_{w,j,in} \right) \Delta\tau_j \right\} \frac{1}{\sum_j \Delta\tau_j}, \tag{10}$$

where $\dot{m}_w$ is the cooling water mass flow, $c_{p,w}$ is the specific heat capacity of the cooling water, and $T_{w,in}$ and $T_{w,out}$ are the cooling water inlet and outlet temperature, respectively.

The heat removed by the air is expressed as

$$q_{air} = \left\{ \sum_j \dot{m}_{air,j} \left[c_{p,air} \left(T_{air,j,out} - T_{air,j,in} \right) + h_{fg} \left(x_{j,out} - x_{j,in} \right) + \right. \right.$$
$$\left. \left. + c_{p,wv} \left(x_{j,out} T_{air,j,out} - x_{j,in} T_{air,j,in} \right) \right] \Delta\tau_j \right\} \frac{1}{\sum_j \Delta\tau_j}, \tag{11}$$

where $\dot{m}_{air}$ is the air mass flow, $c_{p,air}$ and $c_{p,wv}$ are the specific heat capacities of the air and the water vapor, $T_{air,in}$ and $T_{air,out}$ are the air inlet and outlet temperature, x_{in} and x_{out} are humidity of the inlet and the outlet air, and h_{fg} is heat of evaporation.

The average heat flow from the fermentor to the environment in the observed time interval $\Delta\tau$ is

$$q_{loss} = \left\{ \sum_j q_{loss,j} \Delta\tau_j \right\} \frac{1}{\sum_j \Delta\tau_j}. \tag{12}$$

The average heat flow resulting from the action of the mixer in the observed time interval $\Delta\tau$ is

$$P_m = \left\{ \sum_j P_{m,j} \Delta\tau_j \right\} \frac{1}{\sum_j \Delta\tau_j}. \tag{13}$$

The average change in the temperature of the broth ΔT_b in the specified time interval $\Delta\tau$ is

$$\Delta T_b = \left\{ \sum_j \Delta T_{b,j} \Delta\tau_j \right\} \frac{1}{\sum_j \Delta\tau_j}. \tag{14}$$

Considering equations (10) – (14), the reaction heat flow q_r in the time interval $\Delta\tau$ can be calculated with the energy balance equation (8).

RESULTS AND DISCUSSION

Some process parameters during industrial fermentation

Figure 1 shows the progress of some process parameters during the 24 hours industrial fermentation of bacitracin. In figure 1 the connection between the biomass concentration and the oxygen consumption can be seen, whereby the broth temperature is regulated to ensure favourable conditions for microbiological activity.

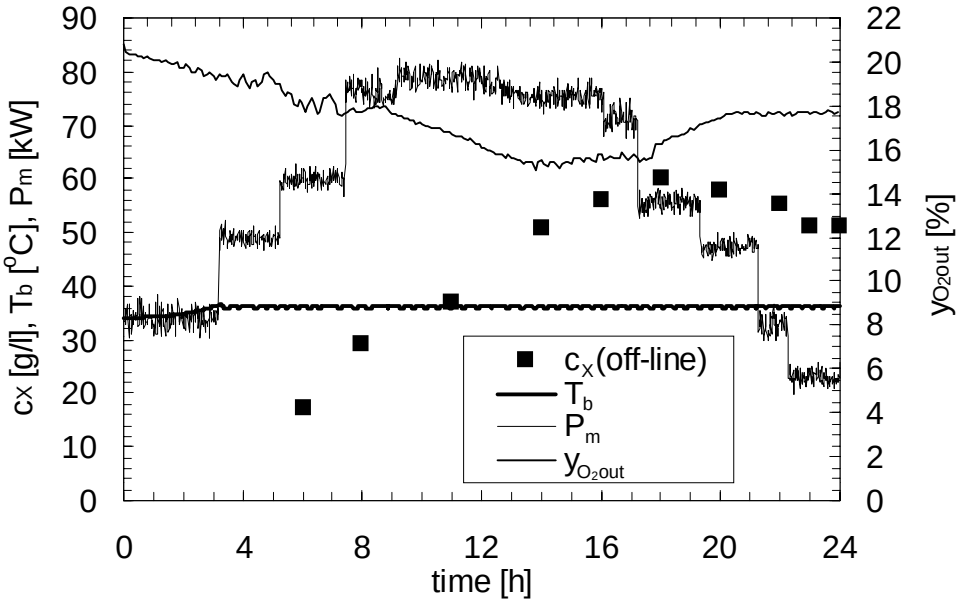


Figure 1 Process parameters during the 24 hours fermentation of bacitracin.

Oxygen uptake rate and reaction heat flow

Figure 2 illustrates the variations of *OUR* under the assumption of a 20.96 % oxygen ratio in air entering the fermentor and the variations of q_r during the 24 hours bacitracin fermentation.

From figure 2 it is clear that there is a high level of correlation between *OUR* and q_r with the correlation coefficient 0.991. An excessively high level of correlation between

measured variables can lead to a divergence of parameter estimates in the model, or render impossible the reconstruction of required state variables.

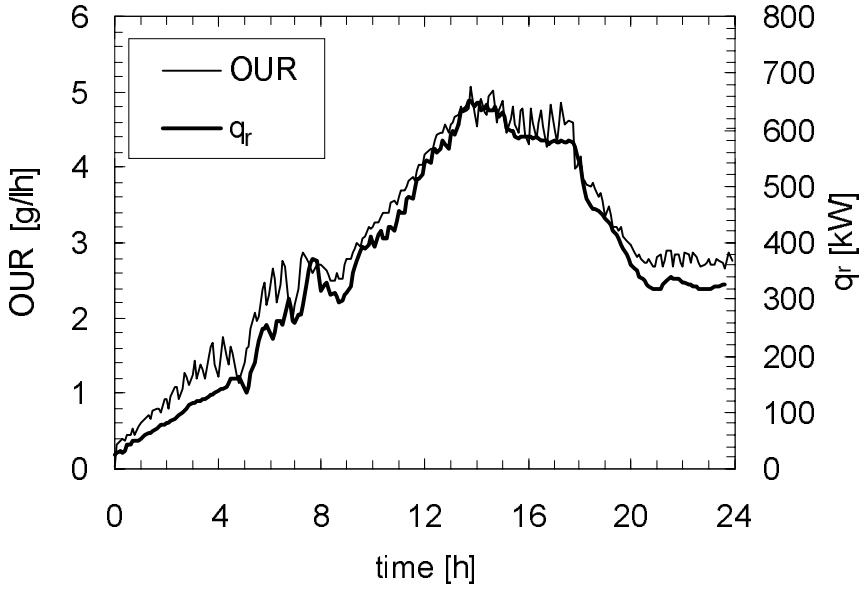


Figure 2 Oxygen uptake rate, *OUR*, and reaction heat flow, q_r.

On the other side, the high level of correlation between *OUR* and q_r enables estimation of specific growth rate μ with the same model, where either OUR or q_r can be used as an input signal. If in equations (4) to (6) OUR is replaced with q_r, two mutual independent software sensors with mutual independent measured input signals have been obtained for real time monitoring of the specific growth rate and biomass concentration during the bacitracin fermentation, which are important indicators of the progress of the industrial fermentation process.

Specific growth rate

Adjustable parameters of the model are average value of the forgetting factor, assessment of the measurement noise and sampling interval, which have to be determined empirically. The estimation algorithm is started up, together with the fermentation process, with the non-recursive least squares method, which ensures good starting values for the non-recursive algorithm and enables consistent prediction of the specific growth rate already after the second hour of the fermentation.

Figure 3 shows the specific growth rates, estimated on-line with O*UR* and q_r as the measurable input signals.

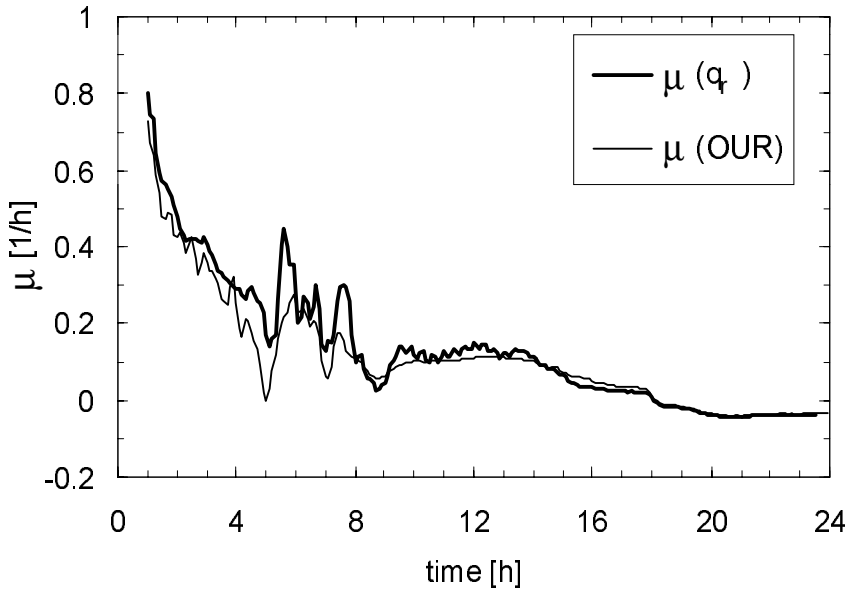


Figure 3 Specific growth rate μ, estimated on the base of measurements of *OUR* and q_r; $d\tau$ = 0.1 h.

The estimates of the specific growth rate converge between the 2nd and 4th hour of the process. After this the estimates consistently follow the development of the process including the characteristic minimums, which occur approximately at the 5th and 7th hours. After the 8th hour, when the production of the secondary metabolites begins, μ becomes settled, except during the technologically important interval at about the end of the 18th hour when the concentration of the biomass begins to drop.

Biomass concentration

Biomass concentration $c_X(\tau)$ can be obtained by integrating the first term of equation (1)

$$c_X(n) = c_X(n-1)exp[\mu(n)\Delta\tau]. \qquad (15)$$

Equation (15) is recursive and requires an accurate value of the biomass concentration at the start of the calculation. Determination of the starting values of the c_X and fine tuning of the estimator, if necessary, is described in[12]. Figure 4 shows the

comparison of the off-line measured biomass concentrations with the estimated biomass concentrations, based on the independently measured input signals *OUR* and q_r.

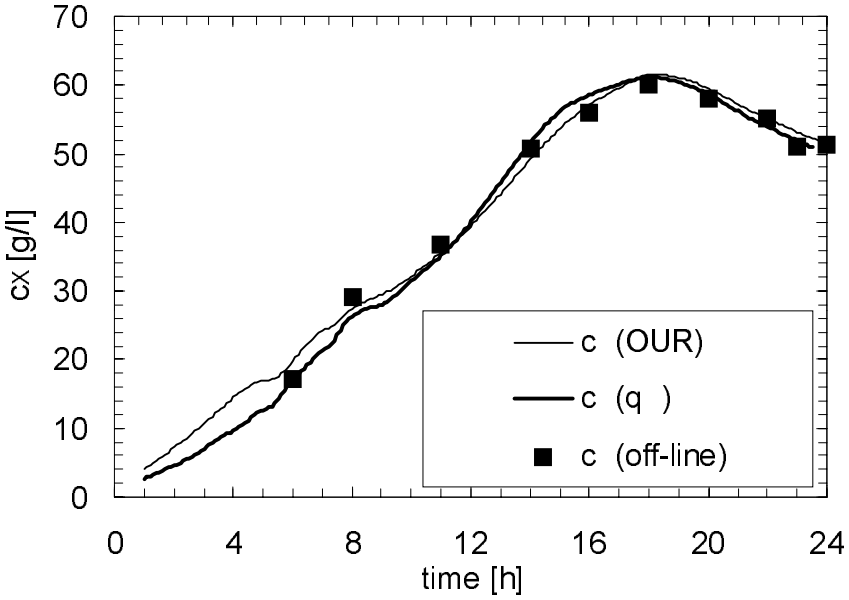


Figure 4 Comparison of the on-line estimates of the biomass concentration in the broth c_X, based on the measurements of *OUR* and q_r, with the off-line measurements, determined with the 'spread plate count' method during the bacitracin production in the 80 m^3 industrial fermentor.

The estimations of the biomass concentration in the broth during the whole, 24 hours industrial bacitracin fermentation, only rarely drop out of the accuracy range of the off-line measurements, which is ± 10 % using the 'spread plate count' method. Good agreement with the off-line measured values indicates promising possibilities for using such sensors when monitoring and optimising the industrial production of bacitracin.

Simulation of disturbances in the process

Developed model enables simulation of some irregularities, which can occur in the industrial fermentation process and have causes in the deteriorated process parameters. As an example, various changes in the air mass flow were simulated, which influence *OUR*, and, as consequence, the specific growth rate and biomass concentration in the broth.

Four different changes in the air mass flow were generated, which are described and enumerated in table 1. Enumeration in table 1 is consistent with the series marking in the legends of the figures 5 to 8.

Table 1 Simulated disturbances in the air mass flow.

enumeration, mark	description
1	Undisturbed.
2	At the 10th hour, the air mass flow dropped for 30 % for 6 min and then returned to normal.
3	At the 10th hour, the air mass flow dropped for 30 % for 12 min and then returned to normal.
4	At the 10th hour, the air mass flow had decreased in 30 min for 30 % and then stayed at this value.
5	At the 10th hour, the air mass flow had decreased for 30 % in 30 min and then increased for 30 % in 30 min. After the 11th hour, the air mass flow was normal again.

All four disturbances in air mass flow are shown in figure 5, together with measured, "normal" series, which is marked with number 1. Transitions between the states during the changes 4 and 5 are sinusoidal. *OUR* was calculated with the equation (7) on the assumption, that the *OUR* is dependent only on the air mass flow. However, because of the complexity of the fermentation process, the microbiological activity is not dependent only on one process parameter. As the measurements of the interrelated dependence of the air mass flow, the oxygen concentration in the exhaust gas and the microbiological activity were not available, the above assumption was used to test capabilities of the developed estimator to detect irregularities in the process. *OUR*'s are shown in figure 6.

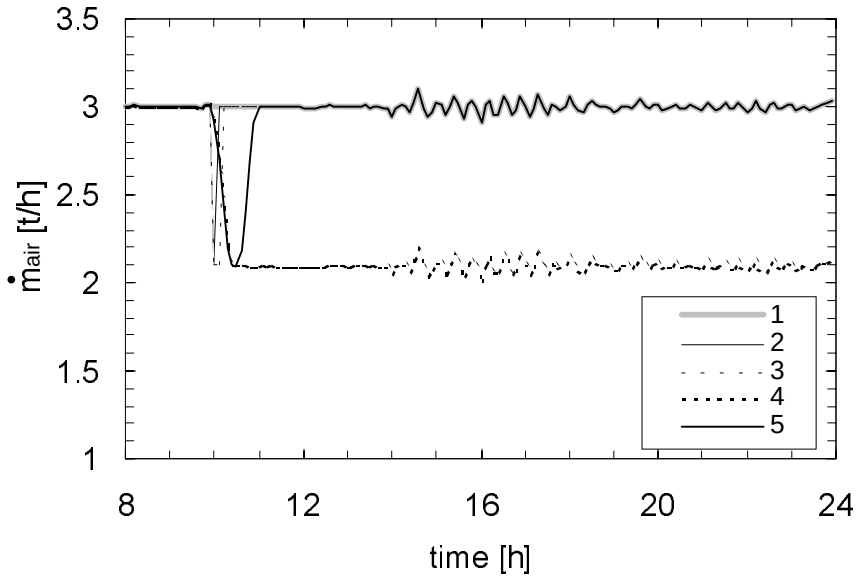
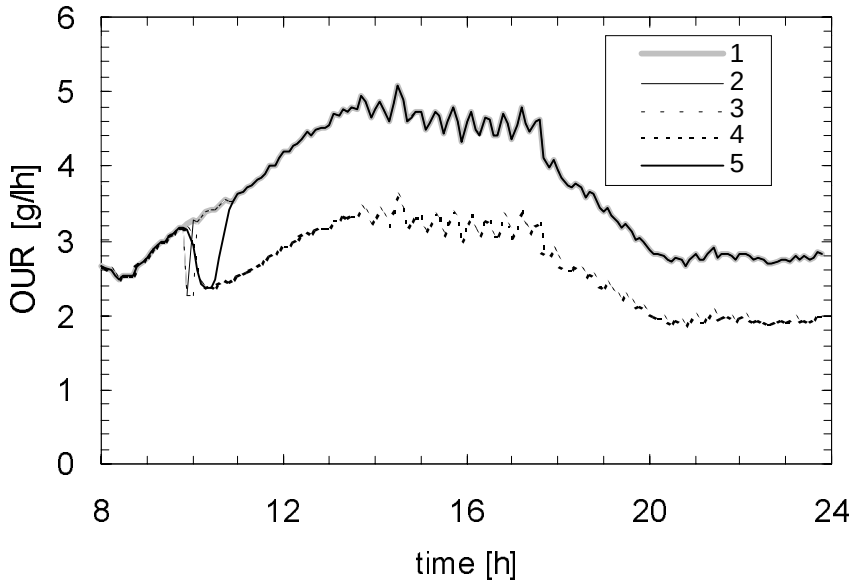


Figure 5 Changes in the air mass flow, all started at the 10 th hour of the process.
Figure 6 *OUR*'s at the various changes in the air mass flow.



In figures 7 and 8, estimated specific growth rates and biomass concentrations for various changes in air mass flow are shown. It can be seen that any change in optimally set air mass flow cause decrease in biomass concentration in the broth, which has direct influence on the bacitracin production. All changes were simulated to start already at the 10th hour of the process to show how an early deterioration of the process parameters can

influence the whole process. It is clear, that the permanent decrease in air mass flow for 30 % results in the lowest quality of the fermentation. More interesting is the capability of the model to characterise very short disturbances in air mass flow (changes 2 and 3) as the cause for lower production. Similar deteriorations are possible in the industrial fermentation (for example, cut-off of the compressors) and are known to have very negative consequences for the production.

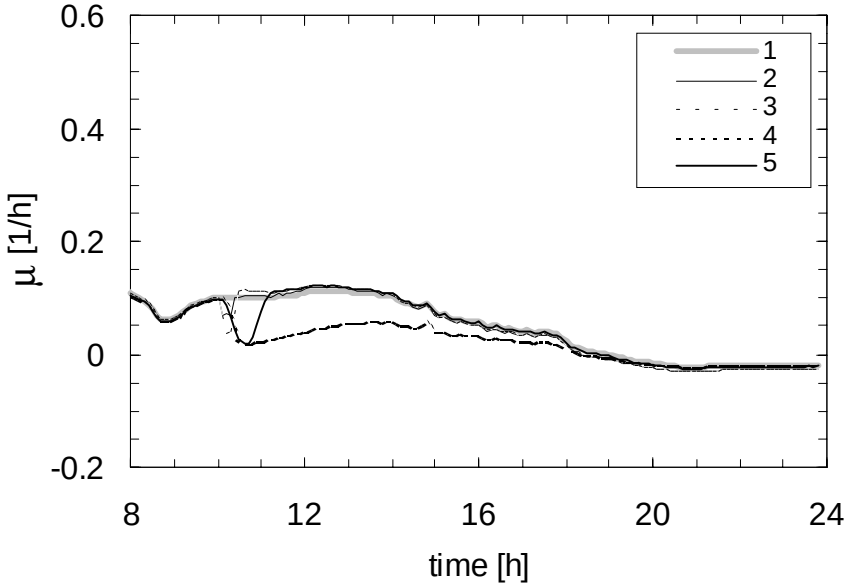


Figure 7 Specific growth rates at the various deteriorations.

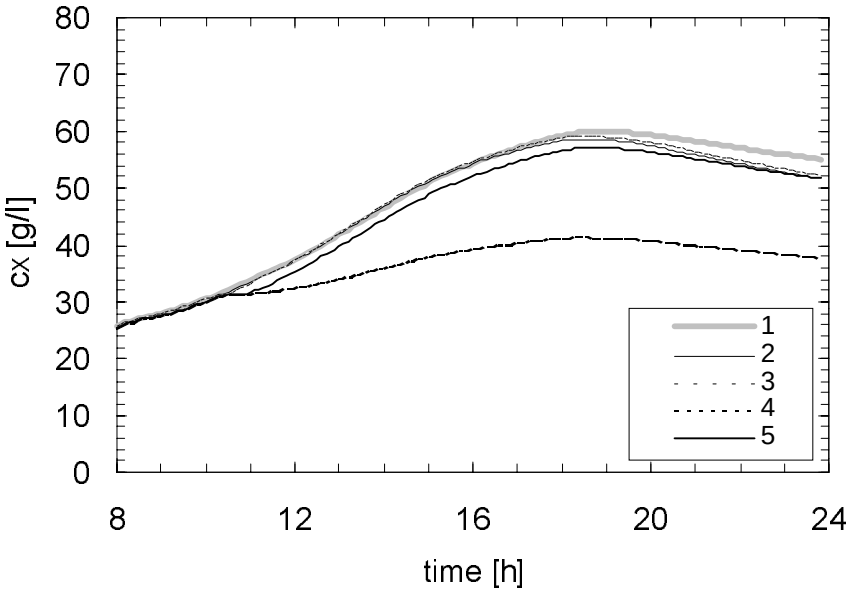


Figure 8 Biomass concentration at the various deteriorations.

The simulations shown consider oxygen concentration in the exhaust gas not to change with the changes in the air gas flow rate and don't consider very complicated interrelation between physical and biological sub-processes. On the other hand, the treated disturbances are hard to be measured during the industrial fermentation process and should be detected before the broth become unusable. Simulation results show that the developed estimator is capable to detect various deteriorations during the fermentation and is useful tool for supervision and control, which enhances reliability of the industrial process.

CONCLUSIONS

During the industrial bacitracin fermentation process in an 80 m^3 bioreactor, specific growth rate and biomass concentration in the broth were estimated in real time. Estimator requires on-line measurement of either oxygen uptake rate or reaction heat flow. Oxygen uptake rate were determined indirectly, with the measurement of the oxygen concentration in the exhaust gas of the fermentor. Reaction heat flow was determined on the basis of the energy balance equation and requires on-line measurement of large number of parameters of the process. Estimator used was recursive least squares model, which was upgraded with the time-varying forgetting factor.

Real time estimation of both estimators is in good agreement with the off-line measured biomass concentration and can unbiased and consistent follow the fermentation process also during very dynamic process phases. Since the methods used for measuring oxygen concentrations in the exhaust gas and reaction heat flow are mutually independent, independent software sensors were obtained. They provide new information during the fermentation process in real time, enable mutual control and control of the measuring sensor, and, to some extent, enable fault detection in the process. These properties increase the quality and the reliability of the production, which is of key importance in industrial fermentation.

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POVZETEK

Na osnovi merjenja kisika v odduhu ali reakcijskega toplotnega toka se je v realnem času določevala specifična hitrost rasti in koncentracija biomase pri fermentaciji bacitracina v 80 m^3 bioreaktorju. Uporabljeni enoparametrični linearni rekurzivni model najmanjših kvadratov s spremenljivim faktorjem pozabljanja dobro sledi nelinearenemu in, v nekaterih fazah procesa, zelo dinamičnemu procesu fermentacije in omogoča simulacijo motenj v industrijskem procesu. Ocenjeni parametri se dobro skladajo z off-line meritvami in predstavljajo nove koristne informacije, ki omogočajo boljši nadzor industrijskega procesa.