

## Mathematical Modelling and Variable Structure Control Systems for Fed-Batch Fermentation of *Escherichia coli*

O. Georgieva\*, I. Hristozov, T. Pencheva, St. Tzonkov, and B. Hitzmann\*\*

Centre of Biomedical Engineering- Bulgarian Academy of Sciences  
105, Acad. G. Bonchev Str., ph. ++359 2 979 3648, Sofia 1113, Bulgaria  
E-mail: olympia@clbme.bas.bg

\*\*Institut für Technische Chemie, Universität Hannover  
Callinstr. 3, ph. 0049-511-762-2963, 30167 Hannover, Germany

Original scientific paper  
Received: November 4, 2002  
Accepted: July 1, 2003

In this paper some problems of the mathematical modelling and high control algorithms of feed-batch fermentation process of *Escherichia coli* are considered. An unstructured mathematical model for *E. coli* feed-batch fermentation is developed on the base of FIA measurements. Methodology for the models' parameters estimation is proposed on the basis of nonlinear optimization methods. The model accurately predicts the process of cultivation of *E. coli*. On the base of the obtained model high control algorithms, such as conventional variable structure systems, variable structure systems with boundary layer and fuzzy variable structure systems, are elaborated. Glucose control strategy of feed-batch cultivation of *E. coli*, according to peculiarities of FIA, is presented to establish small glucose concentration to prevent the accumulation of growth inhibiting metabolites. The presented control algorithms enable to control the glucose concentration at a set point of 0.1 g l<sup>-1</sup>.

*Keywords:*

Fermentation process, modelling, high quality control

### Introduction

Cultivation of recombinant microorganisms, e.g. *Escherichia coli*, in many cases is the only economical way to produce pharmaceutical biochemicals such as interleukins, insulin, interferons, enzymes and growth factors. The fermentation processes are known as very complex and their modeling is rather time consuming. However, it is neither necessary nor desirable to construct comprehensive mechanistic process models that can describe the system in all possible situations with high accuracy. In order to optimize a real biotechnological production process, the model should describe those aspects of the process that significantly affect the process performance.

Many mathematical models have been proposed for fermentations but just a few have been used to optimize industrial fermentations.<sup>1–4</sup> In this paper a simple mathematical model of a typical fed-batch fermentation of *E. coli* with high productivity of biomass, substrate consumption, and acetate production is proposed.

To maximize the volumetric productivities of bacterial cultures it is important to grow *E. coli* to high cell concentration. The use of fed-batch cultivation in the fermentation industry takes advantage

of the fact that residual substrate concentration could be maintained at a very low level in such system. Glucose is one of the most frequent used substrate for the cultivation of microorganisms. A low residual level of substrate could be advantageous in:

- Removing repressive effects of rapidly utilized carbon sources and maintaining conditions in the culture within the aeration capacity of the fermentor.
- Avoiding the toxic effects of a medium component.

Some control loops are required to provide the conditions for optimal growth of microorganisms. For fermentation processes different control loops are realized for the control of pH, temperature, and dissolved oxygen, as well as for the control of volume and anti foam. These control systems are already implemented in modern bioreactors. The production of acetate, which inhibits the biomass production during the growth of *E. coli*, is a result from the high glucose concentration as a carbon source. A direct closed loop control of glucose feeding based on the glucose measurements is not reported yet. The main reason is the difficulty of measuring the substrate concentration in a fast and reliable way.

Flow injection analysis systems (FIA) with a biosensor is used for the monitoring of glucose during the bacterial cultivation process as a fast and reli-

\*Corresponding author

able technique.<sup>5</sup> The FIA system consists of ordinary FIA components, however, two injection valves are employed. An enzyme solution, glucose oxidase (GOD), which can easily be detected, is used to convert the analyte to a substance. This is an opposite of usual biosensor FIA systems, which utilize immobilized enzymes. Using the first injection valve 35  $\mu\text{l}$  of an enzyme solution will be injected into the carrier stream. In this segment, employing the second injection, 17  $\mu\text{l}$  sample solution, which contains the cells also, is injected. Assuming plug flow, the sample segment is placed in the middle of the enzyme solution segment. Both are mixed by the typical dispersion of FIA. Due to the conversion of glucose by GOD some oxygen is consumed. However, the organisms, which come with the sample solution, also consume oxygen during this process. As a consequence, the total consumed glucose is measured with an oxygen electrode. A separate measurement cycle without injection of GOD is performed to determine how much glucose has been consumed by the glucose conversion and how much by the organisms. Only sample solution containing cells is injected. Therefore, during such a measurement oxygen is consumed only by the organisms. The difference of the measured values of both cycles gives the oxygen consumed by the enzyme reaction. Due to the fact that the oxygen consumption of the cells varies very slowly, three successive cycles of second type are performed, before a cycle of new type is activated. However, although the FIA-systems will provide their measurement results faster than all other process analyzers, the delay time of their measurements is usually larger than 5 minutes and will increase during a cultivation run. But the measurement delay time of 5 to 10 minutes increases the substrate control problem significantly.

High control algorithms, such as variable structure systems and its modifications, namely variable structure systems with boundary layer and fuzzy variable structure systems, are elaborated to get over these difficulties. These high control algorithms are presented for fed-batch fermentation of *E. coli* on the base of a mathematical model, obtained with using of FIA measurements.

### Fermentation conditions and measurements

The cultivation of *E. coli* MC4110 is performed in a 2l bioreactor (Bioengineering, Switzerland), using mineral medium, in the Institut für Technische Chemie, Universität Hannover. Before inoculation glucose concentration of 2.5 g l<sup>-1</sup> is established in the medium. In the feeding solution there is 100 g l<sup>-1</sup> of glucose. The initial liquid vo-

lume is 1350 ml, pH is controlled at 6.8, and temperature is kept constant at 35 °C. The aeration flow rate is kept at 275 l h<sup>-1</sup> air. The stirrer speed is 900 rpm at start. It is increased in steps of 100 rpm after 11 h and at the end it is 1500 rpm. The oxygen is controlled at about 35 %.

### Off-line analysis

Determination samples of about 10 ml are taken roughly every hour for off-line glucose measurements as well as biomass and acetate concentration. The off-line measurements are performed using a Yellow Springs Analysers (Yellow Springs Instruments, USA).

### On-line analysis

For on-line glucose determination a FIA system has been employed using two pumps (ACCU FM40, SciLog, USA) for a continuous sample and carrier flow rate. The continuous-discrete extended Kalman filter is used to reduce the measurement noise.

### Process model

The model of fed-batch fermentation of *E. coli* is based on the following assumptions:

- The bioreactor is completely mixed.
- The main products are biomass, water and, under some conditions, acetate.
- The substrate glucose is mainly consumed oxidatively and its consumption can be described by Monod kinetics.
- The acetate production rate is assumed to be directly proportional to the formation of biomass.
- Variation in the growth rate, acetate production and substrate consumption do not significantly change the elemental composition of biomass, thus balanced growth conditions are only assumed.
- Quantities, e.g. temperature, pH, pO<sub>2</sub> are controlled at their individual constant set points.

The rates of cell growth, glucose consumption and acetate formation in *E. coli* fermentation are commonly described according to the mass balance equation as follows:

$$\frac{d\gamma_X}{dt} = \mu \gamma_X - D \gamma_X \quad (1)$$

$$\frac{d\gamma_S}{dt} = -\frac{1}{Y_{s/x}} \mu \gamma_X + D(\gamma_{in} - \gamma_S) \quad (2)$$

$$\frac{d\gamma_{Ac}}{dt} = \frac{1}{Y_{Ac/X}} \mu \gamma_X + D \gamma_{As} \quad (3)$$

$$\frac{dV}{dt} = Q \quad (4)$$

$$\mu = \mu_{\max} \frac{\gamma_S}{K_s + \gamma_S} \quad (5)$$

The rate of increasing of biomass is correlated to the specific growth rate and the biomass concentration. The specific growth rate is generally assumed to be a function of three quantities: the mass concentration of limiting substrate, the maximum growth rate and a saturation constant  $K_s$ . The value for  $K_s$  is generally very low. Maximum specific growth rates have considerable industrial importance. The  $\mu_{\max}$  depends on the organism and fermentation conditions. The specific growth rates for *E. coli* fermentation<sup>6</sup> vary between 0.09–0.61 h<sup>-1</sup>.

### Estimation of the models parameters

For the modelling of cultivation of *Escherichia coli* experimental data obtained in the Institut für Technische Chemie, Universität Hannover, are used for global models' parameter estimation. From whole experimental data just the off-line measurements of biomass, substrate (glucose) and acetate are used.

The data used for model identification are not filtered because of the feed rate performance, which can be seen in Fig. 1.

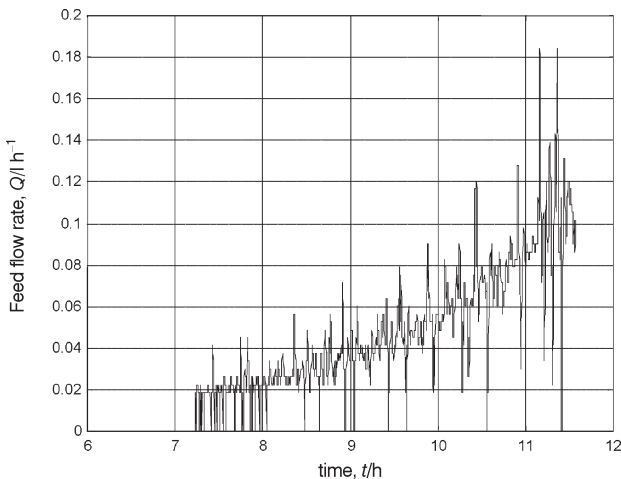


Fig. 1 – Feed flow rate

The estimation of the global models' parameters is made using MATLAB Optimization Toolbox procedures. The Nelder-Mead simplex (direct search) optimization method is used. The method is based on multidimensional unconstrained nonlinear minimization (Nelder-Mead).

To simplify the quantity estimation, it is assumed that quantities  $\mu_{\max}$ ,  $K_s$  and  $Y_{S/X}$  in the Eq. (1)

and (2) are estimated at the first step. The yield  $Y_{A/X}$  in the Eq. (3) is estimated at the second step, while previous coefficients are constants. The optimization criterion is defined as a modelling error means square deviation between the model output and the corresponding data obtained during the fermentation. Therefore, the optimization criteria are  $J_1$  (for the first step) and  $J_2$  (for second step) and they are presented as follows:

$$J_1 = c_1 \sum (\gamma_X - \gamma_X^*)^2 + c_2 \sum (\gamma_S - \gamma_S^*)^2 \quad (6)$$

$$J_2 = \sum (\gamma_{Ac} - \gamma_{Ac}^*)^2 \quad (7)$$

where  $\gamma_X$ ,  $\gamma_S$  and  $\gamma_A$  are experimental data;  $\gamma_X^*$ ,  $\gamma_S^*$  and  $\gamma_A^*$  are model data.

The estimated values of parameters are presented in Table 1.

Table 1

Parameters	Estimated value
$\mu_{\max}$	0.59 h <sup>-1</sup>
$K_s$	0.045 g l <sup>-1</sup>
$Y_{S/X}$	0.5 g g <sup>-1</sup>
$Y_{A/X}$	68.97 g g <sup>-1</sup>

The values of mass coefficients in (6) and optimization criteria (6) and (7) are:

$$c_1 = 1, c_2 = 50,$$

$$J_1 = 16.6917, J_2 = 0.0081.$$

Both the real fermentation trajectories and the simulated ones for the fermentation are presented in Fig. 2, Fig 3 and Fig. 4, respectively for biomass, substrate and acetate.

As can be seen in Figs. 2, 3 and 4, the obtained model with these values of estimated parameters adequately describe and accurately predict the dynamic behaviour of the process of cultivation of *E. coli*.

### Process control

The following characteristics of FIA system are taken into account when the control algorithms are synthesized:

– time of measurements: 1 min = 0.0167 h;

– delay: 45 s = 0.0125 h;

– the passing through the 4<sup>th</sup> pick is considered.

Due to specific peculiarities of FIA,<sup>5</sup> at every 4<sup>th</sup> pick there is no direct measurement of glucose. So,

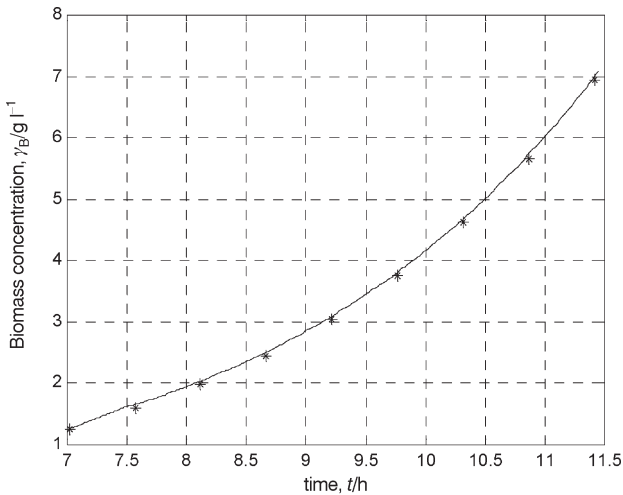


Fig. 2 – Experimental (\*) and model (–) data for biomass concentration

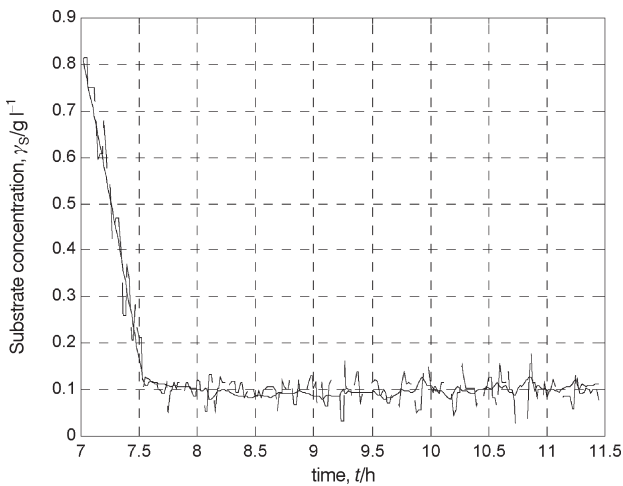


Fig. 3 – Experimental (...) and model (–) data for substrate concentration

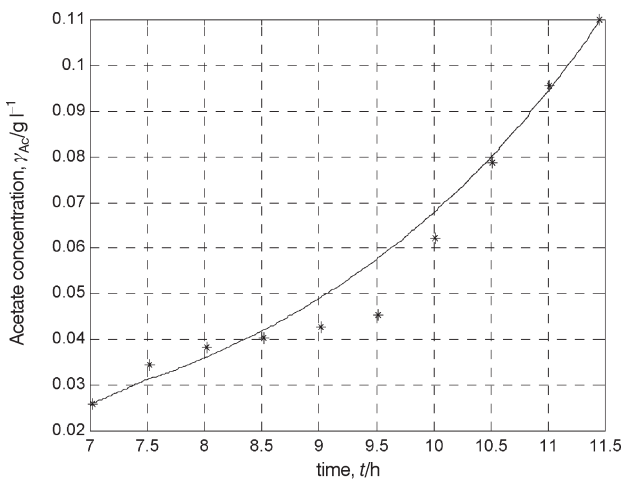


Fig. 4 – Experimental (\*) and model (–) data for acetate concentration

in order to synthesize some control algorithms, this pick has to be missed.

Some control algorithms, which enable the control of the glucose concentration at a set point of  $0.1 \text{ g l}^{-1}$ , have been developed for the fed-batch fermentation of *E. coli*.

The basic aim of control is the concentration of substrate to be kept at some desired value. In this case the desired substrate feed flow rate  $Q$  can be obtained from the Eq. (2):

$$Q = \frac{q_{S,des} \gamma_X V}{(\gamma_{c,in} - \gamma_{S,des})} \quad (8)$$

Conventional variable structure system, variable structure system with boundary layer, and fuzzy variable structure system, are presented here to predict the feeding profile, which ensures the substrate to be kept at the set point. Variable structure systems (VSS) are defined as a class control systems, in which both the structure and parameters are changed by preliminary determined logic law in dependence on the dynamic status of the system. Generally, the control law is constructed in such way, that if the substrate concentration is above its desired value then the aim of the control is to decrease the substrate concentration. Then  $Q$  is obtained from (8):

$$Q_{des} = (1 - \alpha) Q \quad (9)$$

When the substrate concentration is equal to or below its desired value then the aim of the control is to increase the substrate concentration. Then  $Q$  is again obtained from (8):

$$Q_{des} = (1 + \alpha) Q. \quad (10)$$

### Variable structure control system in sliding mode

The VSS in sliding mode (SM) have the most widespread application because of the positive qualities they acquire. If the tracking error is presented as:

$$e = \gamma_S(t) - \gamma_{S,des}(t), \quad (11)$$

the switching surface is defined as:

$$S = e + \lambda \dot{e}. \quad (12)$$

The phase paths of the previous and the new structure should be directed to the switching surface  $S$ . The tracking problem  $\gamma_S(t) - \gamma_{S,des}(t)$  could be considered as a state vector remaining on the sliding surface  $S(x,t) = 0$  for all  $t \geq 0$ . To provide the movement stability it is necessary that the multiplication of switching surface and its derivatives to be negative. In this way the dynamic behaviour describing point is switching with high frequency and it is sliding on the  $s$  to the origin of coordinates. The control law<sup>7</sup> in this case can be obtained from Eq. (10), where

$$\alpha = -c \cdot \text{sgn}(S) \quad (13)$$

The dependence on control variable in sliding mode and the distance between system state and switching line is shown in the Fig. 5. Both the substrate concentration and the control variable feed rate are presented in Fig. 6 and Fig. 7 in the case of 20 % white noise in the output and when constants have values  $\lambda = 0.001$ ,  $c = 0.1$  and therefore  $\alpha = \pm 0.1$ .

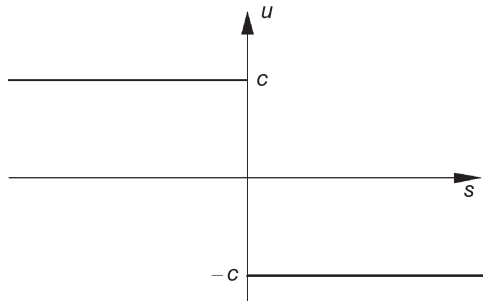


Fig. 5 – Dependence on control variable in sliding mode

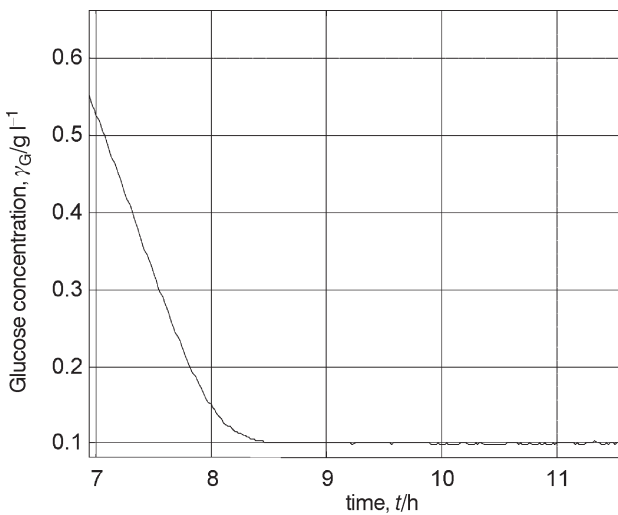


Fig. 6 – Variation of glucose concentration in sliding mode control

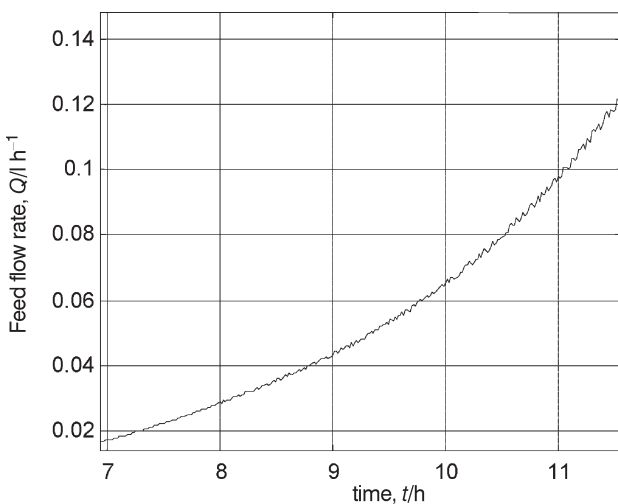


Fig. 7 – Control variable in sliding mode control

VSS in SM have the following advantages:

- quasi-invariety to the outside disturbances and parameter uncertainties;
- to use the bound permissible coefficients in closed loop systems;
- linearising of the description and decreasing of the system dynamic order;
- easy realization of control algorithm in the technical and program terms.

VSS in SM could overcome the parameter uncertainties in some degree. A big disadvantage of VSS in SM is the drastic changes of the control variable (Fig. 7). Therefore, the application of conventional VSS for fermentation process control is restricted because of the control object sensibility to drastic changes of control variable. One possibility to get over this problem is the including of boundary layer near the switching line.

#### Variable structure control system with boundary layer

The including of boundary layer near the switching line requires the upper bounds of the model uncertainties to be known and stability and high performance of the controlled system to be guaranteed. The boundary layer with width  $\Phi$  can be included in the control law Eq. (13)<sup>7</sup> by the substitution of the function  $\text{sgn}(S)$  with  $\text{sat}(S/\Phi)$ :

$$\alpha = -c \cdot \text{sat}\left(\frac{S}{\Phi}\right) \quad (14)$$

$$\text{sat}\left(\frac{S}{\Phi}\right) = \begin{cases} \frac{S}{\Phi} & \text{where } \left|\frac{S}{\Phi}\right| < 1 \\ \text{sgn}\left(\frac{S}{\Phi}\right) & \text{where } \left|\frac{S}{\Phi}\right| \geq 1 \end{cases} \quad (15)$$

When the system is within the boundary layer, the control variable is proportional to the distance between current system state and switching line. Therefore, when the distance between system state and switching line is small, the value of control variable is also small. The dependence on control variable in sliding mode with boundary layer and the distance between system state and switching line is shown in the Fig. 8. Both the substrate concentration and the control variable feed rate are presented in Fig. 9 and Fig. 10. Results are achieved at the same values for the white noise in the output and coefficient  $c$  (as in SM) and when the width of boundary layer has a value  $\Phi = 0.5$ .

The smooth control behaviour (Fig. 10) is achieved when the boundary layer is introduced near the switching line and the frequent changes of control variable sign in the closeness of switching

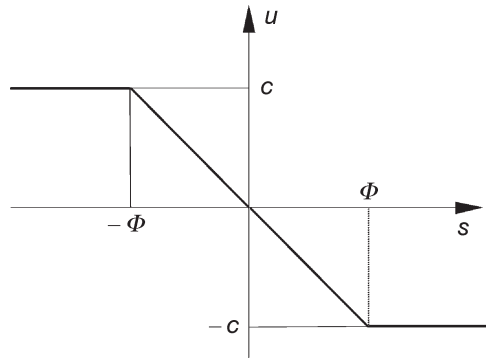


Fig. 8 – Dependence on control variable in sliding mode with boundary layer

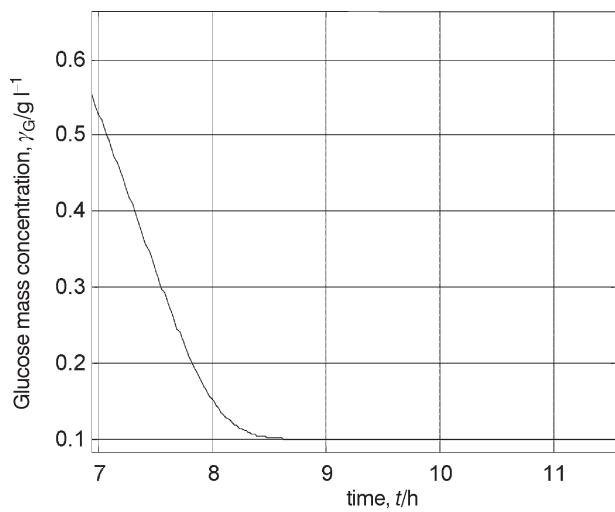


Fig. 9 – Variation of glucose concentration in sliding mode control with boundary layer

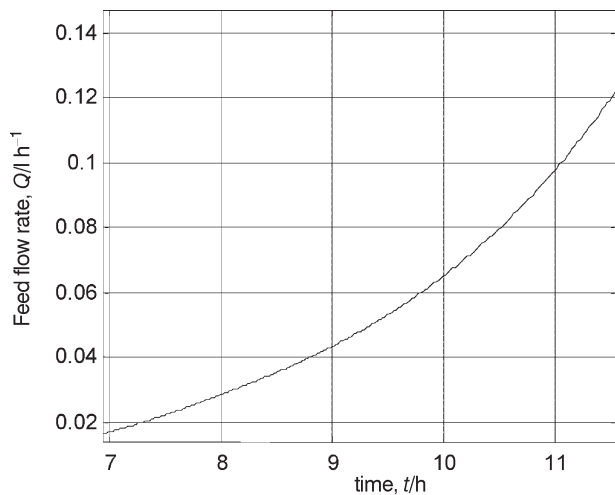


Fig. 10 – Control variable in sliding mode control with boundary layer

line are got over. The remaining of system within this layer is ensured, while the advantages of sliding mode control in VSS are saved.

**Fuzzy variable structure control system**

The control law for fuzzy variable control system for fed-batch process can be presented again by Eq. (10), where

$$\alpha = -c_{fuz}(|S|) \cdot \text{sgn}(S) \quad (16)$$

For the calculation of  $c_{fuz}$  controller type Takagi-Sugeno is synthesized.<sup>7</sup> The crisp value for  $c_{fuz}$  is obtained by defuzzyfication method of weighted average. Fig. 11 shows the dependence on fuzzy control value and distance between system state and switching line. Both the substrate concentration and the control variable feed rate are presented in Fig. 12 and Fig. 13.

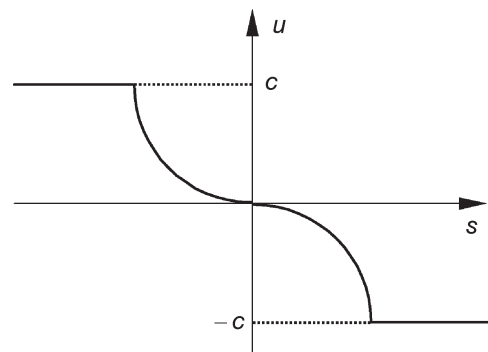


Fig. 11 – Dependence on fuzzy control variable in sliding mode

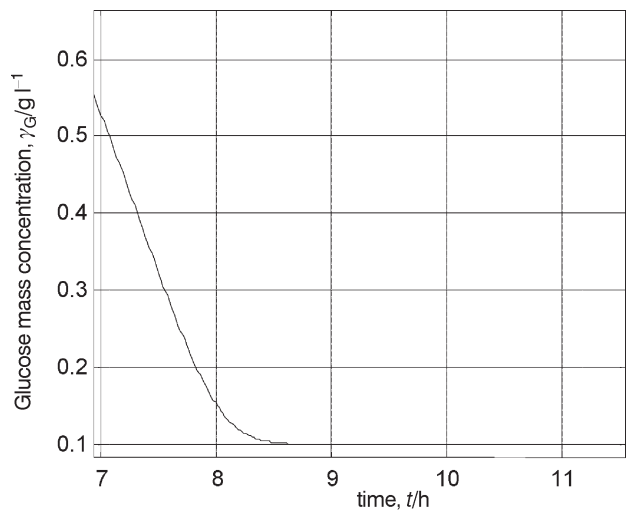


Fig. 12 – Variation of glucose concentration in fuzzy sliding mode control

Fuzzy variable control systems give smoother control (Fig. 13), while the advantages of SM control are saved. The application of fuzzy logic control in the SM control leads to:

- removing of chattering effects;
- significantly decreases of input control variables' magnitudes;

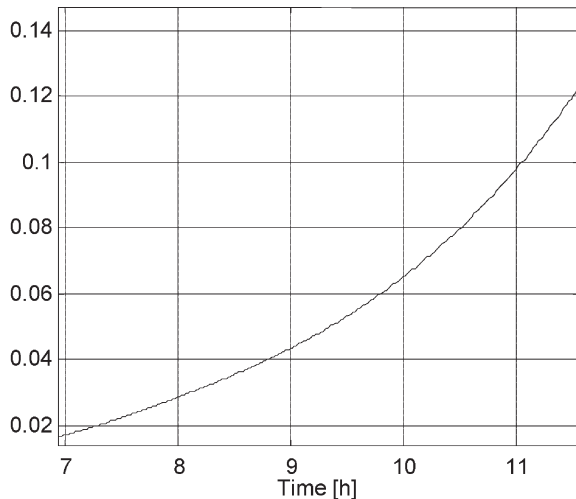


Fig. 13 – Control variable in fuzzy sliding mode control

- improvement of the system performance;
- better qualitative and quantitative indexes.

These advantages set the combined techniques of VSS with fuzzy logic control over the conventional VSS.

The presented high control algorithms lead to smoother feeding profile (Figs. 7, 10, 13) in comparison with the feeding rate, obtained on the basis of extended Kalman filter (Fig. 1). Moreover, the feeding rate profile has been improved on each following control algorithm. The smoothest profile is obtained when fuzzy logic control is implemented in VSS (Fig. 13).

## Analysis and conclusions

An unstructured model has been developed on the basis of experimental data for fed-batch fermentation of *Escherichia coli*, obtained by fast glucose measurement using FIA. The obtained model parameters' values have been estimated by nonlinear optimization methods, simulation studies, and expert knowledge about the main internal variables. The mathematical model describes and predicts successfully the variation of biomass and acetate production and glucose consumption. On the basis of obtained model of the process some high control algorithms are elaborated. Conventional variable structure systems, variable structure systems with boundary layer and fuzzy variable structure systems are presented for control of *E. coli* fed-batch fermentation. The control algorithms are developed according to the peculiarities of FIA. A certain disadvantage of VSS in sliding mode, namely drastic changes of the control variable, is overcome by including the boundary layer or fuzzy logic into the control law. Their application removes chattering effects, significantly de-

creases the input control variables' magnitudes, and improves the system performance. Better qualitative and quantitative indexes set the combined techniques of VSS with boundary layer and fuzzy logic control over the conventional VSS.

## ACKNOWLEDGEMENTS

This work is partially supported from DFG Project N<sup>o</sup> 113/109.

## List of symbols

- $\gamma_X$  – mass concentration of biomass, g l<sup>-1</sup>
- $\gamma_S$  – mass concentration of substrate (glucose), g l<sup>-1</sup>
- $\gamma_{Sin}$  – mass concentration of the feeding solution, g l<sup>-1</sup>
- $\gamma_{Ac}$  – mass concentration of acetate, g l<sup>-1</sup>
- $Q$  – feeding flow rate, l h<sup>-1</sup>
- $V$  – bioreactor volume, l
- $\mu$  – specific growth rate, h<sup>-1</sup>
- $\mu_{max}$  – maximum specific growth rate, h<sup>-1</sup>
- $K_S$  – saturation constant, g l<sup>-1</sup>
- $Y_{S/X}, Y_{A/X}$  – yields, g g<sup>-1</sup>
- $q_s$  – specific rate of substrate utilization, h<sup>-1</sup>
- $D$  – dilution rate  $Q/V$ , h<sup>-1</sup>
- $t$  – time

## Dimensionless symbols

- $J_i$  – optimization criteria
- $c_i$  – weight coefficients
- $e$  – tracking error
- $S$  – switching surface
- $\Phi$  – width of boundary layer
- $\alpha, \lambda, \epsilon$  coefficients

## Superscripts and subscripts

- des – desired value
- fuz – fuzzy value

## References

1. Cockshott A. R., Bogle I. D. L., Bioprocess Engineering, **20** (1999) 83.
2. Galvanauskas V., Simutis R., Volk N., Lubbert A., Bioprocess Engineering, **18** (1998), 227.
3. Standbary P. F., Whitaker A., Principles of Fermentation Technology, Pergamon Press, 1984.
4. Royce P. N., CRC Crit. Rev. In Biotech., (1993), 13, 117.
5. Schoengarth K., Friehs K., Hitzmann B., Int. Symp. BioPS'98, Sofia, Bulgaria, 1998, pp. IV.5.
6. Crueger W., Grueger D., Biotechnology. A textbook of Industrial Microbiology, Sci. Tech. Inc., 1984.
7. Hristozov I., Pencheva T., Hitzmann B., Int. Symp. BioPS'01, Sofia, Bulgaria, 2001 pp. I.53.

