Adaptive Fed-batch Control of *Escherichia coli* Fermentation for Protein Production

Velislava Lyubenova*, Anastasiya Zlatkova, Maya Ignatova

Department of Mechatronic and Bio/technological Systems
Institute of Robotics, Bulgarian Academy of Sciences
Acad. G. Bonchev Str., Bl. 2, P.O. Box 79
E-mails: v_lubenova@hotmail.com, dr666chase@gmail.com, maya.ign@gmail.com

**Corresponding author*

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Abstract: *A new adaptive linearizing control algorithm that stabilizes the carbon source concentration in a desired value is proposed. This algorithm is applied to recombinant protein production by Escherichia coli. A model for control of the investigated process is derived. The model identification is made based on experimental data of the batch phase of the process. The operating model includes two sub-models. Each of them describes one of the two physiological states through which the process passes. Switching from one model to another depends on the sign of a key parameter obtained from the acetate measurements. A cascade scheme of software sensors for the estimation of two biomass growth rates included in the structure of the proposed control algorithm is derived. Simulation studies of the developed closed system have been carried out. The results of the impact of an open-loop control system on the same object are compared.*

Keywords: *Fermentation, Adaptive control, Glucose, Acetate, Recombinant proteins.*

Introduction

Biotechnological processes as highly nonlinear and non-stationary require special methods for their monitoring and control. Modelling of these processes is most often done through mass balance equations, and for the purposes of real-time process monitoring and control, these models usually include concentrations of biomass, main substrates, and products [2]. The aim is to describe the dynamics of the processes as close as possible to the experimental data on the one hand, and on the other hand – their structure should not be too complex, which would make their application difficult. These models have found wide application in monitoring and controlling processes with a single basic product and substrate, [2, 8] as well as in modelling of more complex processes [6, 7].

Another successful approach, close to the previous one concerning mass balance equations and modelled variables, is the state decomposition approach, the so-called functional state approach [21-23]. The concept implementation leads to a process description with simpler and more transparent local models. The approach was originally developed for yeast growth processes. Based on the similarities of the main metabolic pathways of yeast and bacteria, it is applied successfully for the modelling of *Escherichia coli* cultivations [21-23].

Several classes of model-based software sensors (SS) are presented in the literature for on-line monitoring of biotechnological processes [8, 10, 14]. The most popular are mechanistic models derived from first principles [21, 22], classical observers with full knowledge of models, such as

the Luenberger and the Kalman observers [2, 13], asymptotical observers [2, 17], observer-based estimators [2, 8, 11], nonlinear observers [2], adaptive observers [2, 14, 18], etc.

Although the considered above software sensors refer to different bioprocesses, only a few examples concern their implementation to complex ones, described by dynamical models containing several balance equations with complex kinetics.

Regarding the control of these processes, many approaches have been applied in the literature, summarized in the review papers [5, 8, 15, 16]. Some of the more used ones are P (PID) control [6, 24], adaptive control [2, 8, 10], robust adaptive control [18, 19], robust tracking control [3], extremum seeking control [26], nonlinear control [4, 9, 20], multiple nonlinear model adaptive control [27, 28], sliding mode control [25], nonlinear predictive control [1, 4], etc. Recently, in [12], a novel model-based control strategy for the direct control of biomass-specific productivity in recombinant *E. coli* fed-batch processes was established under the usage of nonlinear feedback linearization and the implementation of a two-degrees-of-freedom controller. Future experimental verification will concretize the potential of the control strategy towards the establishment of continuous and more productive recombinant production processes.

Another engineering approach for bioprocess control synthesis is the widely used adaptive linearizing control originally proposed for biotechnological processes in [2, 8], and its modifications and applications can be found in [10, 11, 20].

Escherichia coli is the most widely used host microorganism for recombinant protein (RP) production. These proteins are used to develop enzymatic assays. They serve as valuable tools for investigating cellular responses to stress, and disease situations. RP are used in food production, agriculture, and bioengineering. For example, enzymes can be added to animal feed to increase the nutritional value of feed ingredients, reduce feed and waste management costs, support animal health, improve the environment, etc. The importance of RP has increased rapidly for basic life science research, diagnostic reagents and therapeutic drugs. Their role in biotechnology is irreplaceable.

A high-cell density fed-batch fermentation of *E. coli* is characterized by different physiological states [6, 7] during the cultivation and respectively by multiple growth rates of biomass, which is directly related to the target product. According to the approach in [2], the reaction scheme of such a process could be presented as a set of the following two main reactions (metabolic pathways), which correspond to the process's physiological states:

- Oxidative growth on glucose with specific growth rate μ_1:
$$k_1 S + k_4 O \overset{\mu_1}{\rightarrow} X + k_5 C, \quad (1)$$

- Fermentative growth on glucose with specific growth rate μ_2:
$$k_2 S + k_6 O \overset{\mu_2}{\rightarrow} X + k_7 C + k_3 A, \quad (2)$$

where X, S, A, O and C are concentrations of biomass, glucose, acetate, dissolved oxygen and carbon dioxide in the culture broth, k_1-k_7 – yield coefficients.

The general requirement for process control design is to have available on-line information about all main process variables. The control task from the expert's point of view is to maintain a previously set low value of glucose concentration in the bioreactor.

The purpose is to investigate the possibilities of increasing the efficiency of RP production by applying closed-loop adaptive linearizing control. For this purpose, a general dynamical model (GDM) of the process is derived using the process reaction scheme. The observer of unmeasured variable – biomass as the target product is designed using on-line measured variables – glucose and intermediate product/substrate acetate. The derived control algorithm is investigated by simulations. A comparison of the results from the proposed closed-loop control with a control open-loop algorithm [20] is done. The new algorithm shows better results that are encouraging for its application in laboratory conditions.

Materials and methods

Development of cascade software sensor for the monitoring of the biotechnological process

Operational model

An operational model has been developed [31] based on the scheme of reactions (1), and (2) for the considered process. It consists of two sub-models describing the oxidative growth on glucose – sub-model (3) and oxidative-fermentative growth of biomass on glucose – sub-model (4) as follows:

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ A \end{bmatrix} = \begin{bmatrix} 1 \\ -k_1 \\ 0 \end{bmatrix} \mu_1(t) X - \frac{F_{in,S}}{W}\begin{bmatrix} X \\ S \\ A \end{bmatrix} + \frac{F_{in,S}}{W}\begin{bmatrix} 0 \\ S_{in} \\ 0 \end{bmatrix}, \quad (3)$$

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

where $F_{in,S}$ is the feed rate; S_{in} – glucose concentration in the feed solution; W – the weight of the bioreactor; X, S, and A – concentrations of biomass, glucose and acetate, respectively; μ_1 and μ_2 – specific growth rates. The growth rates $R_{X1} = \mu_1 X$ and $R_{X2} = \mu_2 X$ are considered unknown time-varying parameters that have to be estimated using available on-line information.

Cascade software sensor and stability analysis

A four-step cascade scheme of software sensors has been developed for process monitoring. The general scheme is presented in Fig. 1. Input information includes real-time measurements of acetate (A) and glucose (S) concentrations. The concentrations of these variables can be measured on-line by near-infrared spectroscopy, high-performance liquid chromatography (HPLC) [29, 30], as well as by flow injection analysis (FIA) [20]. The outputs of the monitoring scheme are:

- Acetate production rate, R_{ap} – the first step of the cascade structure;
- Fermentative biomass growth rate, R_{X2} – second step;
- Rate of oxidative growth of the biomass, respectively R_{X1} – third step;
- Biomass concentration, X, and specific rates, μ_1 and μ_2, relating to the oxidative and fermentative growth of biomass – fourth step;

- Rate of glucose consumption, R_S – estimates of this parameter are not included in the cascade structure, but provide valuable information about the process, and can be used in the verification of the obtained estimates of the rates.

The software sensors developed below are derived according to the following assumptions:

- The parameters of models (3) and (4) related to transport dynamics – feeding rate, glucose concentration in the feeding solution and the weight of the culture medium in the bioreactor are known;
- The parameters k_1-k_3, which are inversely proportional to the yield coefficients, are known constants.

If the values of acetate kinetics, R_a, (in the considered case is equal to R_{ap}) are greater than zero, $R_a > 0$, this is an indication that the microorganisms are in a state of oxidative-fermentative growth on glucose. Monitoring of this physiological state involves a software sensor of the rate of acetate production, R_{ap}, which is the input of a software sensor of the rate of fermentative growth on glucose, R_{X2}, which in turn is used as the input of a software sensor of the rate of oxidative growth on glucose, R_{X1}. The obtained estimates for R_{X1} and R_{X2} allow us to calculate biomass concentration estimates and on this basis estimate the specific growth rates μ_1 and μ_2. If the values of acetate kinetics are equal to zero, $R_a = 0$, this is a sign that there is no acetate content in the medium and the growth of the microorganism is oxidative on glucose only.

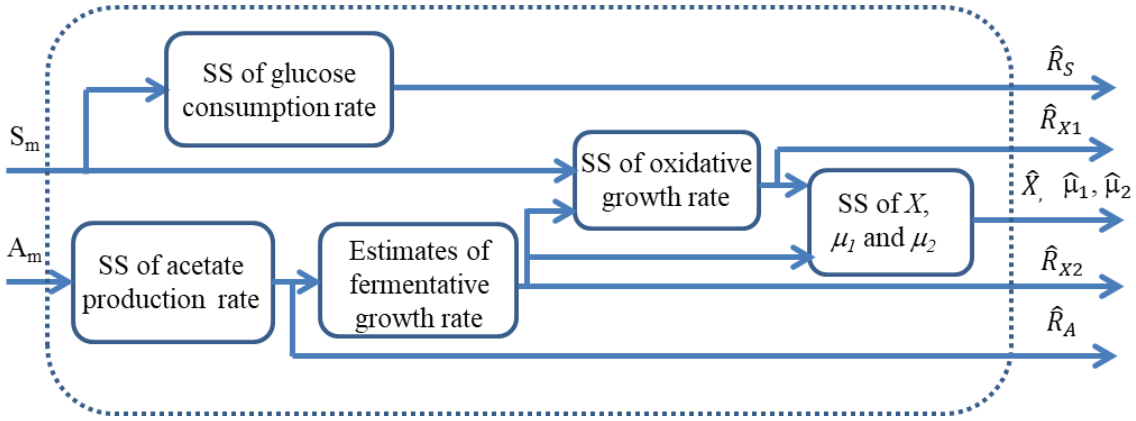


Fig. 1 Scheme of the software sensor

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, $dA/dt \geq 0$. According to the approach proposed in [2], the estimator structure is presented by the following system:

$$\frac{d\hat{A}}{dt} = \hat{R}_{ap} - \frac{F_{in,S}}{W} A_m + w_1(A_m - \hat{A}), \quad (5)$$

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

where $\hat{R}_{ap}$ and $\hat{A}$ are the estimates of acetate production rate (R_{ap}) and acetate concentration (A), respectively; A_m – the measured values of A including white noise (ε_1), $A_m = A + \varepsilon_1$; w_1 and w_2 – estimator tuning parameters which must fulfil the robustness conditions. Eq. (6) is derived assuming that the dynamics of R_{ap} estimates are governed by the difference between the measured and estimated acetate value multiplied by the tuning parameter w_2.

Stability analysis

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

$$\frac{dx}{dt} = Ax + \nu \tag{7}$$

with

$$x = \begin{bmatrix} \tilde{A} \\ \tilde{R}_{ap} \end{bmatrix}, \qquad A = \begin{bmatrix} -w_1 & 1 \\ -w_2 & 0 \end{bmatrix}, \qquad \nu = \begin{bmatrix} -\varepsilon_1 \left(\frac{F_{in,S}}{W} + w_1 \right) \\ -\varepsilon_1 w_2 + \frac{dR_{ap}}{dt} \end{bmatrix}.$$

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

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

Real values are chosen to avoid oscillations in the estimates that do not correspond to the physical indicators related to the estimated parameters.

If it is additionally assumed that there is equality between the eigenvalues as $h_1 = h_2 = h$ with h – a negative constant to ensure the stability of the system (5)-(6), the relation (8a) is rewritten in the form:

$$w_1 = -2h \text{ and } w_2 = w_1^2/4. \tag{8b}$$

The selection of a double eigenvalue has several advantages:

(i) the degrees of freedom of the algorithm is 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.

Estimates of the rate of fermentative biomass growth were obtained in a second step by a mathematical expression which relates R_{X2} to the rate of acetate production, R_{ap}, through the yield factor k_3 as follows:

$$\hat{R}_{X2} = \hat{R}_{ap}/k_3, \tag{9}$$

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

Estimation of the rate of oxidative growth

The third step involves a software sensor of the oxidative growth rate, R_{X1}. It is derived from the dynamic equation for the glucose concentration, S, and the inputs: the measurements for S, as well as the estimates of R_{X2} obtained in (5), (6) and (9). The software sensor is described as follows:

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

$$\frac{d\hat{R}_{X1}}{dt} = w_4 (S_m - \hat{S}), \tag{11}$$

where $\hat{R}_{X1}$ are the estimates of R_{X1}, w_3 and w_4 – parameters of the estimators (10) and (11) with values that are chosen according to the procedure proposed above based on stability analysis and choice of a tuning parameter.

For (10) and (11) the relations between w_3, w_4 and h are as follows:

$$w_3 = -2h \quad \text{and} \quad w_4 = -w_3^2/4k_1. \tag{12}$$

The growth rate, R_{X1}, exists always but the growth rate, R_{X2}, appears when the specific glucose consumption rate is equal to or higher than the so-called critical one [31] and is given below in detail in Eqs. (21) and (22). In the opposite case, $R_{X2} = 0$.

Estimation of biomass concentration and specific rates
This estimation can be done based on the following biomass concentration balance equation, X, from the model (4):

$$\frac{d\hat{X}}{dt} = \hat{R}_{X1} + \hat{R}_{X2} - \frac{F_{in,S}}{W}\hat{X}, \tag{13}$$

where $\hat{R}_{X1}$ and $\hat{R}_{X2}$ are the estimates of the growth rates R_{X1}, and R_{X2}, respectively, derived from algorithms (5), (6), (9) and (12), and $\hat{X}$ are the estimates of biomass concentration X.

The latter allows the calculation of the specific rates μ_1 and μ_2 corresponding to the rates of oxidative R_{X1} and fermentative R_{X2} growth as follows:

$$\hat{\mu}_1 = \hat{R}_{X1}/\hat{X}, \tag{14}$$

$$\hat{\mu}_2 = \hat{R}_{X2}/\hat{X}. \tag{15}$$

Estimation of the rate of glucose consumption
Estimating the rate of glucose consumption from measurements of this substrate, similar to SS (5) and (6), can be accomplished by an observer-based estimator represented by the following system:

$$\frac{d\hat{S}}{dt} = \hat{R}_S - \frac{F_{in,S}}{W}S + \frac{F_{in,S}}{W}S_{in} + w_5(S_m - \hat{S}), \tag{16}$$

$$\frac{d\hat{R}_S}{dt} = w_6(S_m - \hat{S}), \tag{17}$$

where S_m are the measured glucose concentration including white noise (ε_2); $A_m = A + \varepsilon_2$, $\hat{R}_S$ is the estimate of the rate of glucose consumption, which are negative due to the consumption of the substrate; $\widehat{S}$ – estimates of glucose concentration; w_5 and w_6 – the setup parameters for SS (16) and (17), which are chosen depending on a double eigenvalue as follows:

$$w_5 = -2h \text{ and } w_6 = -w_5^2/4. \tag{18}$$

Results and discussion

The simulation investigations of the cascade scheme

Simulation studies of the proposed software sensor scheme have been carried out, based on the developed non-structural models (3), and (4) of the process. Oxidative growth on glucose is represented by the model (3) with the following kinetic model for the specific growth rate μ_1:

$$\mu_1 = q_S/k_1, \tag{19}$$

$$q_S = \frac{q_{S,max}S}{K_S+S}. \tag{20}$$

Oxidative-fermentative growth on glucose is represented by the model (4) with the following kinetic equations for the specific growth rates μ_1 and μ_2:

$$\mu_1 = q_{S,crit}/k_1, \tag{21}$$

$$\mu_2 = (q_S - q_{S,crit})/k_2, \tag{22}$$

with $q_S = \frac{q_{S,max}S}{K_S+S}$ and $q_{S,crit} = \frac{q_{O,max}}{k_{OS}} \frac{K_{i,O}}{K_{i,O}+A}$.

The identification of the kinetic parameters ($q_{S,max}$, K_S, k_1, k_2, k_3, k_{OS}, $q_{O,max}$, $K_{i,O}$) of the models was carried out based on the experimental data up to 10 hours of fermentation, which period could be considered as batch fermentation. All fermentation conditions are presented in [20]. The values of the model parameters, obtained in [31], are given in Table 1.

Table 1. Model parameter values

Parameters	$q_{S,max}$	K_S	k_1	k_2	k_3	k_{OS}	$q_{O,max}$	$K_{i,O}$
Values	2.044	0.148	2.67	20.59	10.77	2.184	0.685	20.198

In Fig. 2 the results obtained by SS (5), (6) are presented. In Fig. 2a the modelled, estimated, and experimental values of acetate concentration are compared. As can be seen, the model data describe the experimental data with good accuracy and match the estimates. By the 18th hour, no acetate had accumulated in the culture medium and the microorganisms were only in a state of oxidative growth on glucose. After this period, acetate production begins and microorganisms grow in conditions of an oxidative-fermentative physiological state. In Fig. 2b modelled and estimated acetate production rate values are compared. Eigenvalues h = –25 and h = –12.5 were tested to investigate the convergence of the estimates and their sensitivity concerning the dR_{ap}/dt derivative. The results have negligible differences for the scores in both cases as can be seen in the figure. The presented estimation errors demonstrate better convergence and accuracy at the higher eigenvalue, especially at the beginning of the fermentation phase, where the maximum relative error reaches 60% for h = –12.5 and 25% for h = –25.

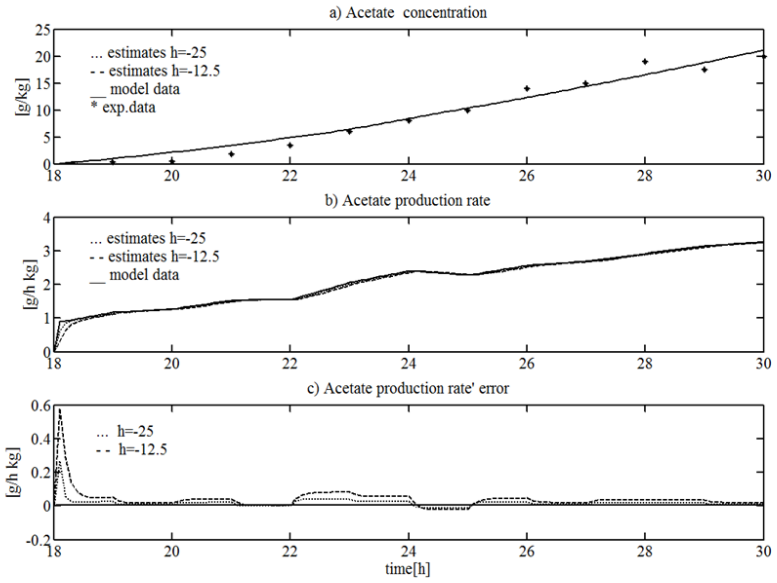


Fig. 2 Estimation of acetate production rate without noise in the measurements for the intermediate metabolite concentration

In Fig. 3, a study was carried out with the same SS with white noise ε added to the acetate measurements, the average deviation of which represented 1% of the average value of the acetate concentration. The simulations were performed using lower eigenvalues compared to the previous study because the sensitivity to noise is very high for h exceeding 5. The maximum estimation errors for both cases (Fig. 3c) are almost twice as high as those in the absence of noise (Fig. 2c), and the averages even more so. Studies have shown that the proposed eigenvalues in the setup are a good compromise between the speed of convergence and sensitivity of the estimates to the perturbations under consideration. A higher eigenvalue is a better choice to tune the estimator, as it gives smaller errors and better convergence to the onset of the fermentative metabolic state, as can be seen in Fig. 3c.

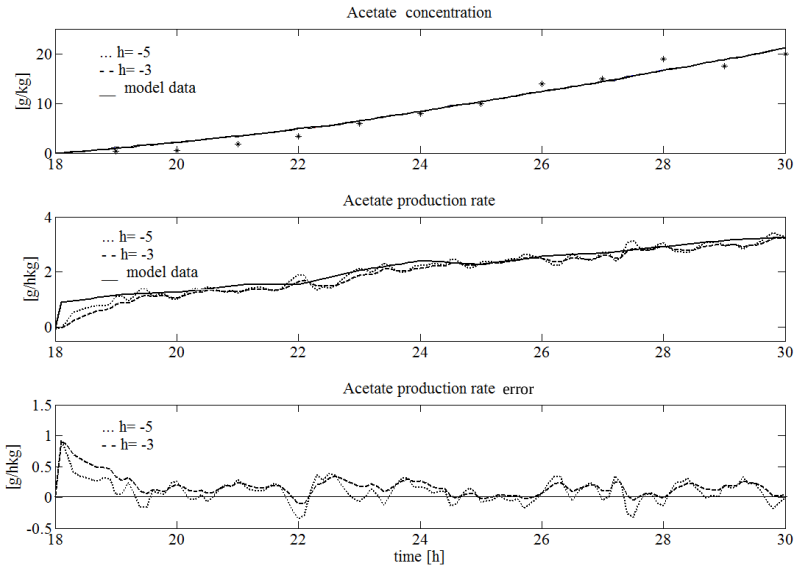


Fig. 3 Estimation of acetate production rate at 1% additive noise in the measurements for intermediate metabolite concentration

Adaptive linearizing control design

The linearizing control design consists of three main steps. The first one is a choice of the input/output model of the closed-loop system. In the case under consideration, the control system input is the feed rate, *F*, and the system output is the glucose concentration, *S*. Hence, the input/output model is presented with:

$$dS/dt = -k_1\hat{R}_{X1} - k_2\hat{R}_{X2} - F_{in,S}/W\, S_m + \left(F_{in,S}/W\right)S_{in}, \quad (23)$$

where the estimates of two biomass growth rates are included.

As a consequence of the specific structure of the GDM, the input/output model is linear concerning the control input (*F*). Hence, the second step is to select a stable reference model of the tracking error, ($S^*(t)$ – $S_m(t)$). A first-order reference model is selected. In the case of stabilisation, $dS^*dt = 0$ because S^* is a constant value, and the reference model can be presented as follows:

$$\lambda(S^* - S_m) = dS/dt, \quad (24)$$

where λ is the controller coefficient. Its value has been chosen so that the equation $\lambda d(S^* - S_m)/dt = 0$ is stable.

The third step is a substitution of the input/output model (23) in the reference one (24):

$$\lambda(S^* - S_m) = -k_1\hat{R}_{X1} - k_2\hat{R}_{X2} - F_{in,S}/W\, S_m + \left(F_{in,S}/W\right)S_{in}. \quad (25)$$

The control algorithm is derived by solving Eq. (24) concerning control input *F*:

$$F = \frac{W(-\lambda(S^* - S_m) + k_1\hat{R}_{X1} + k_2\hat{R}_{X2}}{S_{in} - S_m}. \quad (26)$$

As can be seen, the complete information is available for the calculation of the control action: the estimates from the SS and the values of the yield coefficients from Table 1.

In Fig. 4 the scheme of a closed-loop control system is shown. The value of S^* was picked value of 0.01 and the concentration of glucose in the feed S_{in} is 250 g/l, chosen from the expert's point of view.

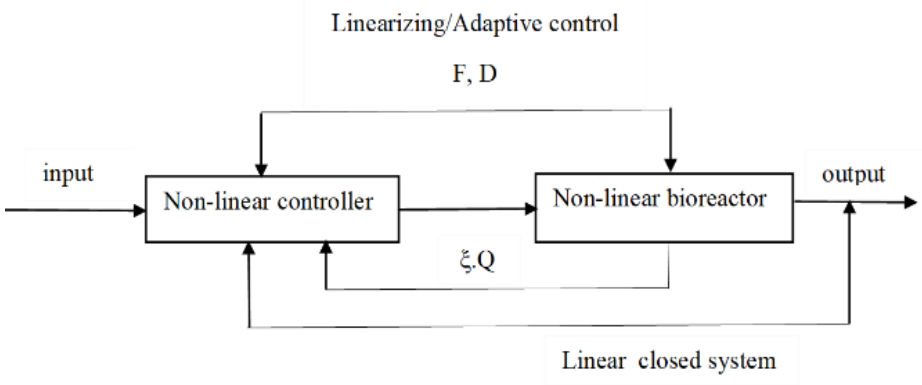
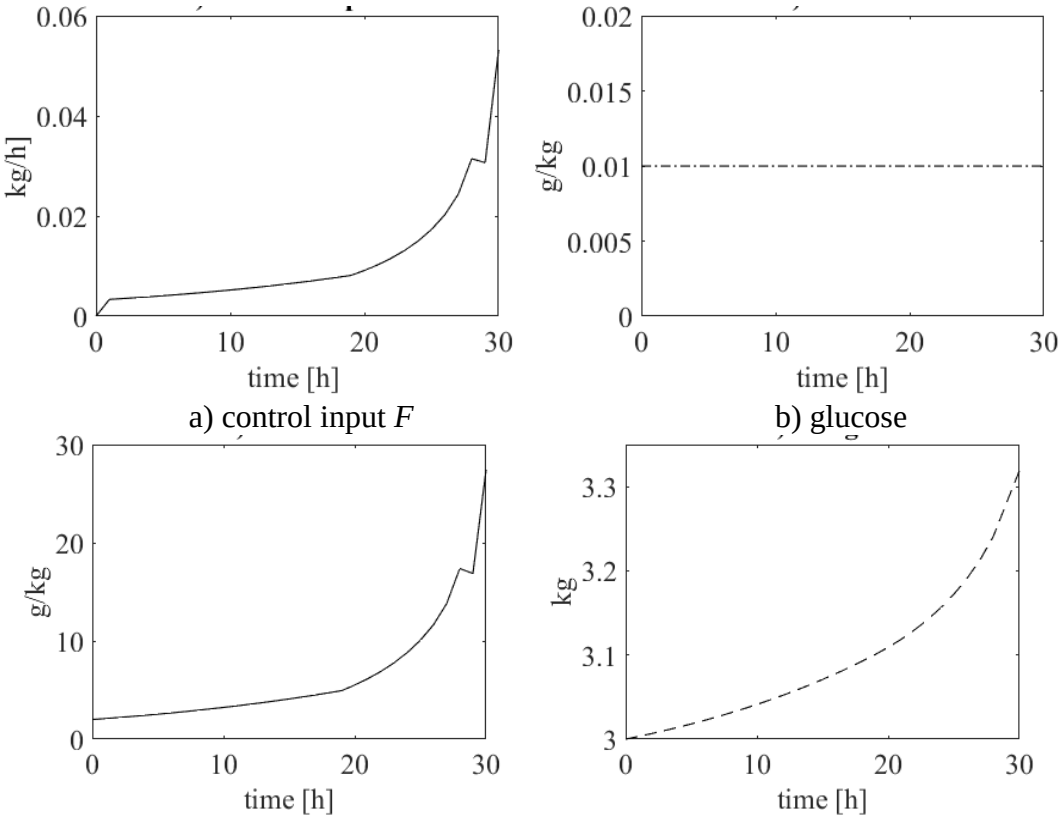


Fig. 4 Control scheme

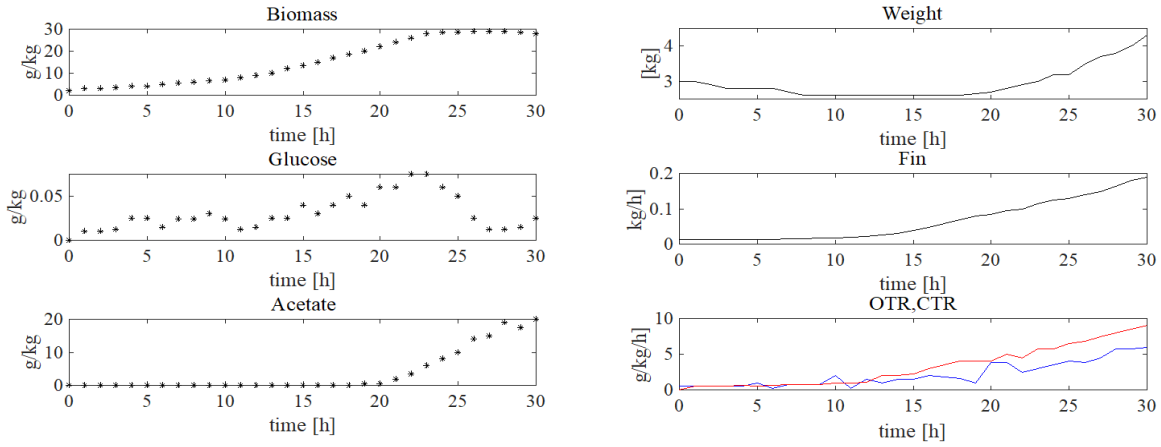
In Figs. 5 and 6, a comparison between two control algorithms is done.



a) control input F b) glucose

c) biomass d) weight

Fig. 5 Linearizing control algorithm investigation



a) b)

Fig. 6 Open-loop control: a) experimental data for the concentrations of biomass, glucose and acetate; b) experimental data for weight, feed rate and oxygen transfer rate (red line) and carbon dioxide transfer rate (blue line)

In Fig. 5 the results of the simulation investigations for the derived adaptive control are presented. They show that the control is turned on at the beginning of the process and up to 30 h the working volume of the bioreactor (5 kg) is not exceeded. In Fig. 6, the results from open-loop control [20] are shown. The control is an exponential function precomputed and applied to the bioreactor.

Maintaining a constant value of S^* can achieve the almost same concentration of biomass (target product) (Fig. 5c) as in Fig. 6a, but it can be noted that the biomass concentration in the proposed control continues to grow until the end of the process (Fig. 5c), while in the other one, retention and decline in biomass concentration after 25 h was observed (Fig. 6a). This decrease may be due to the combination of factors, which are the increased biomass density during the fermentation process and the presence of acetate, which starts to be produced around the 20th hour. These factors inhibit the growth of biomass.

At the same time, the weight at the end of the process is smaller (Fig. 5d), in comparison with Fig. 6b, which leads to better efficiency of the proposed control.

Further research has to be done to investigate the impact of various disturbances on monitoring and control as well as the choice of algorithm‘s tuning parameter before applying this method in laboratory conditions.

Conclusion

A closed-loop control of fed-batch fermentation by *E. coli* is proposed. The derived adaptive linearizing control aims to stabilize the glucose concentration at a previous set value. It is proposed from an expert’s point of view. The process model is identified using the experimental data of the batch part of *E. coli* fermentation. The model is used to design observers of unmeasured two biomass growth rates. The observers are adaptive and included in the structure of the proposed control algorithm to determine the adaptive properties of the closed system.

A comparison between two control algorithms – open-loop and closed-loop control of the same object is realized. The adaptive properties of closed-loop systems are advantageous in controlling non-linear and non-stationary processes such as biotechnological ones.

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Prof. Velislava Lyubenova, D.Sc.
E-mail: v_lyubenova@ir.bas.bg

Velislava Lyubenova is a full Professor at the Institute of Robotics (IR) – Bulgarian Academy of Sciences (BAS). In 2016 she defended her D.Sc. Degree in the field of Monitoring of Biotechnological Processes. At present, she is the Head of the Department "Mechatronic Bio/Technological Systems" at IR – BAS. She has more than 90 published articles in peer-reviewed journals with more than 200 citations. She was a supervisor of 7 international and 5 national projects. Prof. Lyubenova has a few long-term specializations in prestigious scientific institutions in Italy, Portugal and Germany. Her research interests are in modelling, monitoring and control of bioprocesses.

Chief Assist. Prof. Anastasiya Zlatkova, Ph.D.
E-mail: dr666chase@gmail.com

Anastasiya Zlatkova is a Chief Assistant Professor at the IR – BAS. She has a M.Sc. Degree in Industrial Biotechnologies from Sofia University "St. Kliment Ohridski" in 2013. In 2015 she started her Ph.D. thesis in the field of Industrial Automatization. It was successfully defended in 2021. She was a team leader of a project supported by BAS under the "Program for Assistance to the Young Scientists". She has one realized long-term specialization in Braga, Portugal.

Prof. Maya Ignatova, Ph.D.
E-mail: maya.ign@gmail.com

Maya Ignatova graduated in Electrical Engineering, Industrial Automation, Technical University of Sofia (TU – Sofia), Bulgaria (1971) and obtained a Ph.D. Degree in Applied Sciences – Control Systems, TU – Sofia (1979). Since 1991, She is an Associate Professor in Applied Sciences – Biotechnological Process Control Systems at Central Laboratory of Bioinstrumentation and Automation – BAS. Since 2012, she is a Professor in IR – BAS. Prof. Ignatova is an author of more than 120 scientific papers, 2 books and a principal investigator/participant in 26 research contracts and grants. Her main fields of interest are monitoring and control of biotechnological processes.