ISSN 1392 – 124X INFORMATION TECHNOLOGY AND CONTROL, 2006, Vol. 35, No. 4

OPTIMIZATION OF BIOMASS PRODUCTION IN FED-BATCH CULTURE BY FEED AND DILUTION CONTROL ACTIONS

Donatas Levišauskas, Vytautas Galvanauskas, Rimvydas Simutis,

Process Control Department, Kaunas University of Technology
Studentų St. 50, Kaunas, Lithuania

Antanas Žilinskas, Julius Žilinskas

Institute of Mathematics and Informatics
Akademijos St. 4, Vilnius, Lithuania

Abstract. A problem of the model-based optimization of fed-batch cultivation process is solved for two control actions: feed-rate and dilution rate. A goal of optimization is to maximize the yield of cells' biomass at the end of cultivation cycle. Two alternative optimization methods, based on the maximum principle of Pontryagin and the finite dimensional optimization approach, are developed for the problem solving. Numerical example illustrating application of the optimization algorithms is presented and discussed.

1. Introduction

Fed-batch cultivation technique is a mode of bioreactors operation that provides distinct advantages over the other operation modes and has a widespread industrial application [1]. Concentration of substrate in cultivation medium of fed-batch process can be externally manipulated by altering the feed rate. Therefore, an optimal state of microorganisms' culture can be maintained by using the appropriate feed-rate profiles. Optimization of fed-batch cultivation processes is one of relevant problems in biotechnology. Recently the mathematical model-based optimization approach is widely used for development of fed-batch processes for production of new products, as well as for improvement of the established processes [2-7].

In the fed-batch cultivation processes, several factors influence the process state and govern the process behavior [8, 9]. Along with the concentration of substrate, the process is influenced by the products of metabolism that accumulate in cultivation broth and cause an inhibition of cells' growth. The metabolites concentration can be altered by dilution of the cultivation broth with pure water. Therefore, the two control actions can be considered for optimization of fed-batch cultivation processes: feeding with the substrate solution and additional dilution with pure water.

It should be stressed that production of particular biotechnological products, especially in pharmaceutics industry, is subjected to strong technological regulations and improvement of the established processes requires long lasting approval procedure. However, dilution of cultivation medium with pure water at the end of cultivation cycle is not considered to be a principal modification of technological conditions and can be applied, if relevant, without restrictions.

In this work, the two types of practical optimization problems are considered. The first type is related to the development of new fed-batch processes, when no restrictions on the initial technological conditions are imposed. The second type is related to an improvement of the established processes, when initial technological conditions are fixed.

Solutions of similar optimization problems formulated for a single control variable – feeding rate of substrate solution are demonstrated for various fed-batch cultures [2, 3, 5]. Solving of the feed rate optimization problem involving more then one feed rates is investigated in [6]. The usual optimization techniques rest on maximum principle [2-4] or parametric optimization [5-7].

In this work, we used the maximum principle necessary conditions of optimality to develop the two control actions optimization algorithm for mathematical model of generalized structure. An alternative optimization algorithm is developed by applying the parametric optimization approach. A numerical example is presented to evaluate working capacity and efficiency of the proposed algorithms.

2. Formulation of optimization problem

Optimization problem is to maximize a yield of cells' biomass at the end of fed-batch cultivation cycle by manipulating the feed and dilution rates.

Mathematical model of the process refers to the mass balance conditions in bioreactor and has the following generalized structure:

$$\frac{dx}{dt} = \mu(s,x)x - u_1\frac{x}{V} - u_2\frac{x}{V}, \; x(t_o) = x_o, \quad (1)$$

$$\frac{ds}{dt} = -q(s,x)x + u_1\frac{s_f - s}{V} - u_2\frac{s}{V}, \; s(t_o) = s_o, \quad (2)$$

$$\frac{dV_1}{dt} = u_1, \; V_1(t_o) = 0, \; V_1(t_f) = V_{1f}, \quad (3)$$

$$\frac{dV_2}{dt} = u_2, \; V_2(t_o) = 0, \; V_2(t_f) = V_{2f}, \quad (4)$$

$$V = V_0 + V_1 + V_2, \quad (5)$$

where x, s are cells' biomass and substrate concentrations [g/L], respectively; s_f is substrate concentration in feeding solution [g/L]; V_1 is volume of substrate solution [L]; V_2 is volume of water; V is volume of cultivation broth in bioreactor [L]; V_o is initial volume of cultivation broth [L]; μ is specific rate of biomass growth [1/h]; q is specific rate of substrate consumption [g(substrate)/g(biomass)h]; u_1 is supply (flow) rate of substrate solution (manipulated variable), u_2 is supply (flow) rate of water (manipulated variable) [L/h]; t is time [h].
The objective function is

$$J = x(t_f)V(t_f) \rightarrow \max, \quad (6)$$

where t_f is a free terminal time.

The following restrictions are imposed on the manipulated variables:

$$0 \le u_1 \le u_{1,\max}, \quad (7)$$

$$0 \le u_2 \le u_{2,\max}. \quad (8)$$

3. Optimization methods and algorithms

3.1. Application of maximum principle

The maximum principle of Pontryagin provides with the necessary conditions of optimality that must be met along an optimal control trajectory.
Using an integral form of the objective function (6)

$$J = x(t_f)V(t_f) = \int_{t_o}^{t_f} \mu(s,x)xV dt \rightarrow \max, \quad (9)$$

the Hamiltonian takes the following form:

$$\begin{aligned} H = {} & \mu(s,x)x \cdot V + \\ & \lambda_x\left(\mu(s,x)x - u_1\frac{x}{V} - u_2\frac{x}{V}\right) + \\ & \lambda_s\left(-q(s,x)x + u_1\frac{s_f - s}{V} - u_2\frac{s}{V}\right) + \\ & \lambda_{V1}u_1 + \lambda_{V2}u_2 \end{aligned}, \quad (10)$$

where $\lambda_x, \lambda_s, \lambda_V$ are adjoint variables specified by equations

$$\begin{aligned} \frac{d\lambda_x}{dt} = {} & -\frac{\partial H}{\partial x} = -\frac{\partial \mu(s,x)}{\partial x}x \cdot V - \mu(s,x) \cdot V - \\ & \lambda_x\frac{\partial \mu(s,x)}{\partial x}x - \lambda_x\mu(s,x) + \lambda_x\frac{u_1}{V} + \lambda_x\frac{u_2}{V} + , \\ & \lambda_s\frac{\partial q(s,x)}{\partial x}x + \lambda_s q(s,x) \end{aligned} \quad (11)$$

$$\begin{aligned} \frac{d\lambda_s}{dt} = {} & -\frac{\partial H}{\partial s} = -\frac{\partial \mu(s,x)}{\partial s}x \cdot V - \\ & \lambda_x\frac{\partial \mu(s,x)}{\partial s}x + \lambda_s\frac{\partial q(s,x)}{\partial s}x + , \\ & \lambda_s\frac{u_1}{V} + \lambda_s\frac{u_2}{V} \end{aligned} \quad (12)$$

$$\begin{aligned} \frac{d\lambda_{V1}}{dt} = {} & \frac{d\lambda_{V2}}{dt} = -\frac{\partial H}{\partial V_1} = -\frac{\partial H}{\partial V_2} = \\ & -\mu(s,x)x - u_1\lambda_x\frac{x}{V^2} - u_2\lambda_x\frac{x}{V^2} + . \\ & u_1\lambda_s\frac{s_f - s}{V^2} - u_2\lambda_s\frac{s}{V^2} \end{aligned} \quad (13)$$

For process of unfixed duration, along an optimal trajectory the Hamiltonian must be identically zero:

$$\begin{aligned} H = {} & \varphi(s,x,V,\lambda_x,\lambda_s) + \\ & u_1\phi_1(s,x,V,\lambda_x,\lambda_s,\lambda_{V1}) + , \\ & u_2\phi_2(s,x,V,\lambda_x,\lambda_s,\lambda_{V2}) = 0 \end{aligned} \quad (14)$$

where φ is the collection of terms which are not explicit functions of u_1 and u_2,

$$\varphi = \mu(s,x)xV + \lambda_x\mu(s,x)x - \lambda_s q(s,x)x, \quad (15)$$

ϕ_1 is the collected coefficients of u_1,

$$\phi_1 = -\lambda_x\frac{x}{V} + \lambda_s\frac{s_f - s}{V} + \lambda_{V1} \quad (16)$$

and ϕ_2 is the collected coefficients of u_2,

$$\begin{aligned} \phi_2 = {} & -\lambda_x\frac{x}{V} - \lambda_s\frac{s}{V} + \lambda_{V2} = \\ & \phi_1 - \lambda_s\frac{s_f}{V} + \lambda_{V2} - \lambda_{V1} \end{aligned}. \quad (17)$$

Application of the maximum principle to the feed rate optimization is related with the singular control problem, which arises when the control variables enter model equations in a linear manner [10]. It is characteristic of the solutions to the singular control problems that the switching functions ($\phi_1(t), \phi_2(t)$) become identically zero over some finite time intervals. By comparing the equations (16), (17) it can be stated that the switching functions ϕ_1 and ϕ_2 of the respective control variables cannot become identically zero simultaneously, therefore, the singular control intervals of u_1 and u_2, if they appear in an optimal control algorithm, are to be shifted in time.

The state variable trajectory corresponding to singular control must lie on the singular control surfaces ($S_{u1}=0$, $S_{u2}=0$) that can be determined from the necessary conditions of optimality. Along the singular control interval of u_1 ($u_2=0$) condition (14) is satisfied if

$$\varphi \equiv \frac{d^n\varphi}{dt^n} \equiv 0\,,\ n=1,2,... \tag{18}$$

$$\phi_1 \equiv \frac{d^n\phi_1}{dt^n} \equiv 0\,,\ n=1,2,... \tag{19}$$

Similarly, along the singular control interval of u_2 ($u_1=0$) condition (14) is satisfied, if

$$\phi_2 \equiv \frac{d^n\phi_2}{dt^n} \equiv 0\,,\ n=1,2,... \tag{20}$$

simultaneously with the condition (18).

From the equations (13), (16), (17), (19), (20) it follows that along the singular control intervals for u_1 and u_2 the adjoin variables λ_{V1} and λ_{V2} vary according to equations (21) and (22), respectively:

$$\frac{d\lambda_{V1}}{dt} = -\mu(s,x)x - u_1\frac{\lambda_{V1}}{V}\,, \tag{21}$$

$$\frac{d\lambda_{V2}}{dt} = -\mu(s,x)x - u_2\frac{\lambda_{V2}}{V}\,. \tag{22}$$

Initial conditions for the equations (21), (22) are to be chosen to satisfy conditions of transversality:

$$\lambda_{V1}(t_f) = C_{V1}\,, \tag{23}$$

$$\lambda_{V2}(t_f) = C_{V2}\,, \tag{24}$$

where C_{V1}, C_{V2} are some constants, related to the fixed final values of the volumes $V_1(t_f)$, $V_2(t_f)$, respectively.

By combining necessary conditions (18)-(20), state equations (1)-(4), (5) and adjoint variable equations (11)-(13), the variables λ_x, λ_s can be eliminated and the singular control surfaces $S_{u1}=0$ and $S_{u2}=0$ are obtained in a space of the process state variables and adjoint variables λ_{V1} and λ_{V2}, respectively:

$$\begin{aligned} S_{u1} = {} & \frac{\partial\mu}{\partial s}(s_f - s)V - \frac{\partial\mu}{\partial x}xV - \\ & \lambda_x\left(\frac{\partial\mu}{\partial x}x - \frac{\partial\mu}{\partial s}(s_f - s)\right) + \\ & \lambda_s\left(\frac{\partial q}{\partial x}x - \frac{\partial q}{\partial s}(s_f - s)\right) = 0 \end{aligned}\ , \tag{25}$$

where

$$\lambda_x = \lambda_s\frac{s_f - s}{x} + \lambda_{V1}\frac{V}{x}\,, \tag{26}$$

$$\lambda_s = \mu V\frac{x + \lambda_{V1}}{qx - \mu(s_f - s)}\,, \tag{27}$$

λ_{V1} is calculated by integration of the equation (21).

$$\begin{aligned} S_{u2} = {} & -\frac{\partial\mu}{\partial s}sV - \frac{\partial\mu}{\partial x}xV - \\ & \lambda_x\left(\frac{\partial\mu}{\partial x}x + \frac{\partial\mu}{\partial s}s\right) + \lambda_s\left(\frac{\partial q}{\partial x}x + \frac{\partial q}{\partial s}s\right) = 0 \end{aligned}\ , \tag{28}$$

where

$$\lambda_x = -\lambda_s\frac{s}{x} + \lambda_{V2}\frac{V}{x}\,, \tag{29}$$

$$\lambda_s = \mu V\frac{x + \lambda_{V2}}{qx + \mu s}\,, \tag{30}$$

λ_{V2} is calculated by integration of the equation (22).

As optimal boundary conditions (23), (24) are not known in advance, the proper value of λ_{V1} or λ_{V2} at the singular control surface reaching point (initial value for starting integration of equation (21) or (22)) that maximizes performance index (6) can be found by iterative calculation procedure.

The surfaces S_{u1} and S_{u2} are also the singular control switching boundaries since any point of the state space which is not on singular control surfaces must be associated with bang-bang control. With the singular control surfaces it is relatively easy to determine the optimal bang-bang type control actions for process states around S_{u1} and S_{u2} that move the process state towards the singular control surfaces. It should be noted that existence of S_{u1} and S_{u2} does not necessarily imply the both singular controls will be parts of the optimal control.

In this work, we have investigated the optimization problem with unfixed and fixed initial technological conditions. In the first case, dilution of cultivation broth with water can be performed at any time of fed-batch cycle. In the second case, dilution can be performed at the final stage of fed-batch cycle after the feeding substrate is poured into bioreactor.

For the optimization problem with the unfixed initial conditions calculation procedure, based on the necessary conditions of optimality, consists of the following steps:

1. Guess the values of adjoint variables λ_{V1} and λ_{V2} at the singular control surfaces reaching points (initial conditions for equations (21), (22)) and apply extreme values of control variables ($u_1 = 0$ or $u_1 = u_{1\max}$ and $u_2 = 0$ or $u_2 = u_{2\max}$) that move the process state to the singular control surfaces S_{u1} and S_{u2}.
2. By reaching the singular control surfaces, calculate the singular control trajectory that holds the process state on the singular control surfaces. Calculation of $u_{1,\sin g}$ and $u_{2,\sin g}$ is performed by introducing into calculation algorithm the proportional controllers with the gain coefficients $K_{u1} = k_1 \mu x V$, $K_{u2} = k_2 \mu x V$ that manipulate the control actions u_1 and u_2 to keep the conditions (25), (28) (k_1 and k_2 are adjustable parameters of calculation algorithm).
3. Go to step 1, set the other initial values of adjoint variables for equations (21), (22) and repeat the calculation procedure until the maximum value of performance index (9) is gained.

For the fixed initial conditions, optimization procedure consists of the following steps:

1. Guess the value of adjoint variable λ_{V1} at the singular control surface S_{u1} reaching point (initial condition for equation (21)) and apply extreme values of control variable u_1 ($u_1 = 0$ or $u_1 = u_{1\max}$) that moves the process state to the singular control surface.
2. By reaching the singular control surface, calculate the singular control trajectory that holds the process state on the singular control surface. Calculation of $u_{1,\sin g}$ is performed by introducing into calculation algorithm the proportional controller with the gain coefficient $K_{u1} = k_1 \mu x V$ that manipulates the control action u_1 to keep the condition (25).
3. At the end of the singular control interval of u_1 (when the feeding substrate is exhausted), guess the value of adjoint variable λ_{V2} at the singular control surface S_{u2} reaching point (initial condition for equation (22)) and apply extreme values of control variable u_2 ($u_2 = 0$ or $u_2 = u_{2\max}$) that moves the process state to the singular control surface.
4. By reaching the singular control surface, calculate the singular control trajectory that holds the process state on the singular control surface. Calculation of $u_{2,\sin g}$ is performed by introducing into calculation algorithm the proportional controller with the gain coefficient $K_{u2} = k_2 \mu x V$ that manipulates the control action u_2 to keep the condition (28). Dilution period with the $u_2 = u_{2\max}$ or $u_2 = u_{2\sin g}$ is finished, when the water amount for dilution is exhausted.
5. Go to step 1, set the other initial values of adjoint variables for equations (21), (22) and repeat the calculation procedure until the maximum value of performance index (9) is gained.

It should be noticed that the singular control interval for the substrate feeding rate ($u_{1,\sin g}$) always appear in the sequence of the optimal control actions, while appearance of the singular dilution rate ($u_{2,\sin g}$) in the optimal control algorithm is occasional.

3.2. Application of finite dimensional optimization

Methods based on necessary conditions of optimality are helpful to find local maximum of a functional in a feasible region of controls. However, the local maximum can be not unique. We do not have a proof of concavity of the problem (1)-(6), and cannot guarantee that the control defined above corresponds to global maximum. Therefore it seems reasonable to apply alternative methods for searching maxima of the functional of interest *J*. Among great variety of methods of optimal control most general are methods based on reduction of optimal control problems to the problems of finite dimensional mathematical programming [11]. Since local optimization methods normally are applied to the resulting problems of mathematical programming, also in this case local solutions are obtained. However, it is important that local minima different from those defined in previous section can be found.

The simplest mathematical programming problem related to the optimal control problem (1)-(6) is the six dimensional minimization problem where control functions $u_1(t)$ and $u_2(t)$ are of the type *on-off*:

$$u_1(t) = \begin{cases} 0, \ 0 \le t < t_{1\min}, \\ u_1, \ t_{1\min} t \le t_{1\max}, \\ 0, \ t > t_{1\max}, \end{cases}$$

$$u_2(t) = \begin{cases} 0, \ 0 \le t < t_{2\min}, \\ u_2, \ t_{2\min} t \le t_{2\max}, \\ 0, \ t > t_{2\max}, \end{cases}$$

and the optimization variables $t_{1\min}$, u_1, $t_{1\max}$, $t_{2\min}$, u_2, $t_{2\max}$ should satisfy the constraints:

$$0 \le t_{1\min} < t_{1\max}, 0 \le t_{2\min} < t_{2\max},$$
$$0 \le u_1 \le u_{1\max}, 0 \le u_2 \le u_{2\max}.$$

Since the objective function is defined by means of a numerical integration of a system of nonlinear differential equations, its values are computed with some errors. The application of a gradient descent algorithm to such an optimization problem seems not reasonable because numerical estimates of gradients can be too much corrupted by errors in function values. Our previous experience shows that similar problems can be efficiently solved by the Hooke-Jeeves algorithm [12, 13]; therefore this algorithm has been chosen also for the considered problem.

4. Optimization results and discussion

The code for the objective function J optimization algorithms has been written in MATLAB, where the system of differential equations (1)-(5) is numerically integrated using the code ODE45 implementing the algorithm by Dormand-Prince. Simulation of the optimal process was realized in MATLAB/SIMULINK environment.

The discussed optimization algorithms were applied for optimization the process of cells' biomass growth described by mathematical model (1)-(5), in which the specific rates $\mu(s,x)$ and $q(s,x)$ have the following structures:

$$\mu(s,x)=\mu_{\max}\frac{s}{s+K_s}\frac{K_i}{K_i+s}\frac{K_x}{K_x+x}-K_{xx}x \qquad (31)$$

$$q(s,x)=\mu_{\max}\frac{1}{Y_{xs}}\frac{s}{s+K_s}\frac{K_i}{K_i+s}\frac{K_x}{K_x+x}+m \qquad (32)$$

The model (1)–(5), (31), (32) parameter values and boundary conditions of state and control variables are given in Table 1.

Realization of the maximum principle based optimization procedure for the unfixed initial conditions leads to the control action sequence and the state variable trajectories, presented in Figures 1a-e. In Figures 1a, b time profiles of the optimal control actions u_1 and u_2 are presented ($u_1(t)=u_{1,\sin g}$, $5.136\le t\le 18.026$; $u_2(t)=2.0, 0.0\le t\le 3.0$). In Figures 1c, d optimal time trajectories of the state variables x and s are shown, and in Figure 1e augmentation of the performance index J (defined by (9)) is given. The value of the performance index achieved at the end of cultivation process is $J=119.58$.

Table 1. Model parameter values and boundary conditions of state and control variables

Parameter values	Boundary conditions
$\mu_{\max}$ =*0.65* [1/h]	x(0)=*0.1* [g/L]
K_s=*2* [g/L]	s(0)=*20* [g/L]
K_i=*35* [g/L]	V_o=*5* [L]
K_x=*2* [g/L]	V_{1f}=*1* [L]
K_{xx}=*0.000175* [L/gh]	V_{2f}=*6* [L]
Y_{xs}=*0.5* [g/g]	$u_{2\max}=u_{2\max}=2$ [L/h]
m=*0.05* [g/gh]	
s_f=*200* [g/L]	

Alternative solutions of the optimization problem are obtained by using rectangular shape control action profiles and finite dimensional optimization approach. The *on-off* type control functions corresponding to the two local maxima of J ($J=119.04$ and $J=119.21$) found by means of the Hooke-Jeeves algorithm are presented in Figures 2a, b ($u_1(t)=1.0, 2.5078\le t \le 3.5078$; $u_2(t)=2.0, 0.0039\le t\le 2.9375$) and Figures 3a, b ($u_1(t)=1.9844, 0\le t\le 0.5040$; $u_2(t)= 1.9102, 0.4297\le t\le 3.5708$), respectively. The local maxima are found using the initial points defining bang-bang control where the control functions are *on maximum* over the following disjoint neighbor intervals:

$u_1(t)=2.0, 3.0\le t\le 3.5; u_2(t)=2.0, 0\le t\le 3.0$ and

$u_1(t)=2.0, 0\le t\le 0.5; u_2(t)=2.0, 0.5\le t\le 3.5$,

correspondingly; in the first case, the water is fed at maximal rate followed by the feeding of substrate at maximal rate, and in the second case the order of feeding by substrate and water are changed.

The sequence of graphs in Figures 2c–e, 3c–e corresponds to that in Figures 1c–e.

As it follows from the results presented in Figures 1–3, the both optimization approaches give close values of the performance index, in spite of difference in time profiles of the calculated feeding and dilution rates.

The main advantage of the *on-off* type control algorithms is that they can be found by a relatively simple optimization procedure and easy practical implementation of control actions.

Application of the maximum principle requires more efforts to develop optimization algorithm, however the maximum principle based optimal solution gives slightly higher value of performance index and shorter duration of cultivation process. In addition, by applying the maximum principle an optimal solution is obtained in few iterations, while the finite dimensional optimization algorithm requires significantly more iterative calculations (computation time), depending on a number of parameters to be optimized.

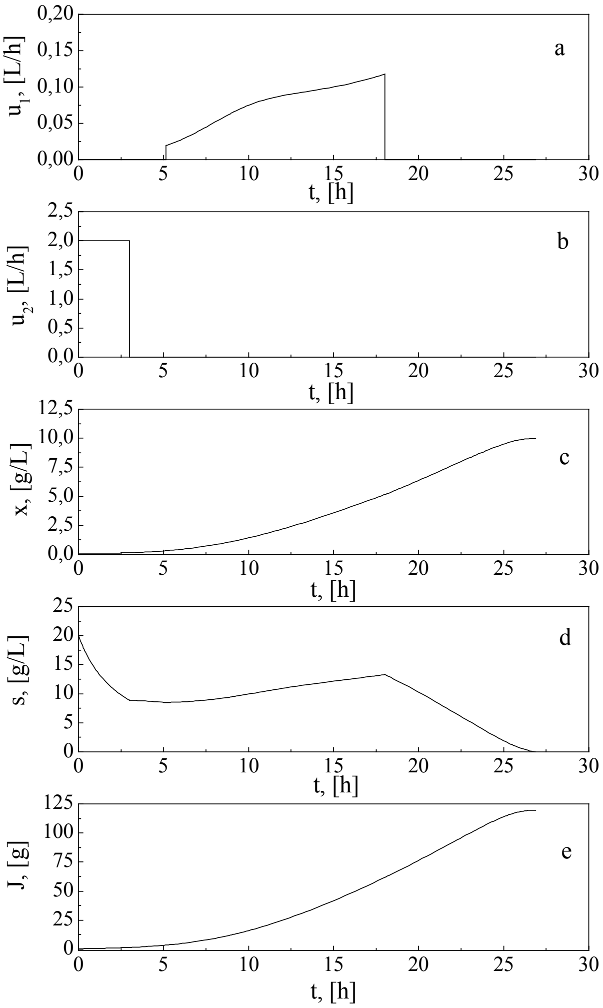

Figure1. Results of process optimization for the unfixed initial technological conditions calculated using the maximum principle based optimization algorithm

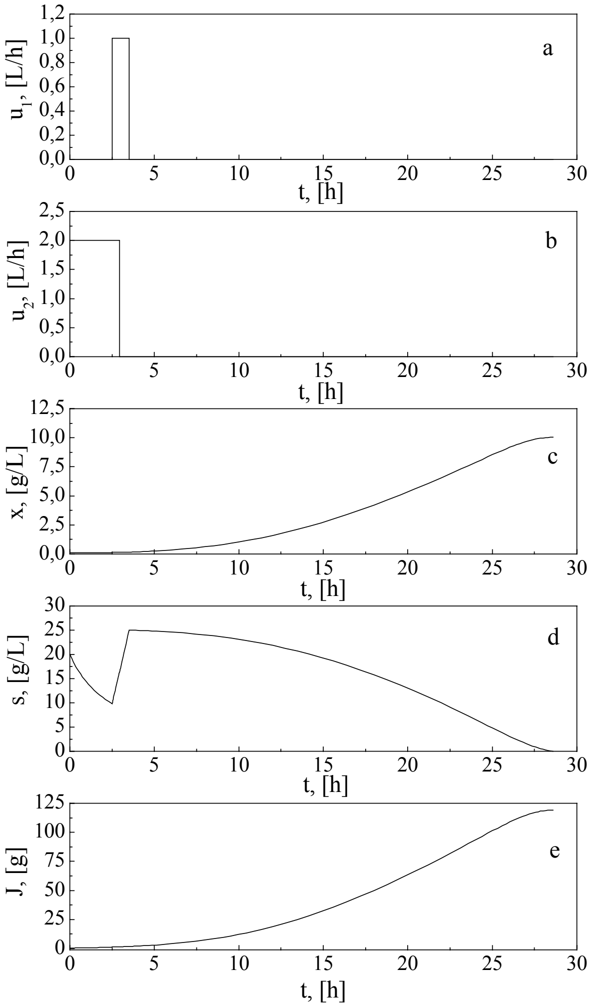

Figure 2. Results of process optimization for the unfixed initial technological conditions calculated using the finite dimensional optimization algorithm

Application of the maximum principle based optimization procedure for a problem with the fixed initial technological conditions gives optimization results presented in Figures 4a–e (solid lines). The calculated control actions ($u_1(t) = u_{1,\sin g}, 13.704 \le t \le 23.247; u_2(t) = 23.247, 0.0 \le t \le 26.247$) are presented in Figures 4a, b. The sequence of the other graphs (Figures 4c–e) corresponds to that in previous figures. Additionally, Figures 4a, c–e present the process optimization results using the feed-rate control only (dotted lines), i.e., when no action of dilution was applied. The value of the performance index obtained by using the dilution control action at final stage of cultivation process totals $J = 115.8$, which is in 9.45 % higher compared with the process at which no dilution action is applied ($J = 105.8$).

5. Conclusions

Two alternative optimization methods, the maximum principle of Pontryagin and finite dimensional optimization approach, are presented for solving a technological problem of the dynamic optimization of fed-batch cultivation process by two control actions: feeding rate and dilution rate. The investigated optimization problem is relevant to fed-batch cultivation processes, in which the process of biomass growth is influenced by metabolic products accumulated in cultivation broth.

The maximum principle based optimization procedure is developed for mathematical model of general structure. The optimization procedure allows obtaining fairly accurate control algorithms, however requires some efforts to derive the specific model-based equations and analytical expressions.

Application of the investigated finite dimensional optimization algorithm allows finding close to optimum control algorithms using predetermined shape control actions. Investigation results demonstrate easy realization of the developed calculation procedures and fast convergence of iterative solutions to optimum.

A numerical example of the optimization algorithms application is presented and discussed.

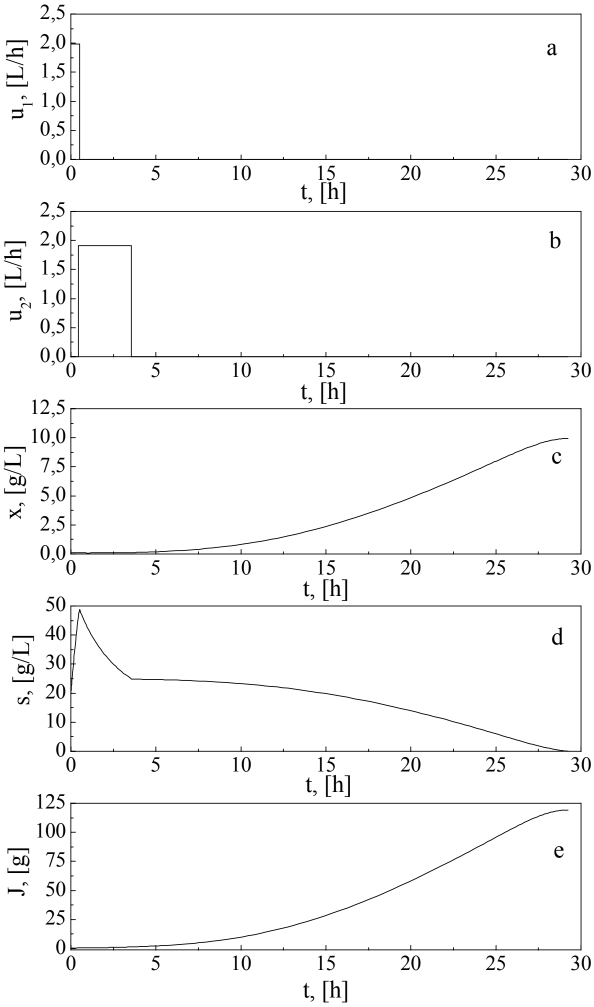

Figure 3. Results of process optimization for the fixed initial technological conditions calculated using the finite dimensional optimization algorithm

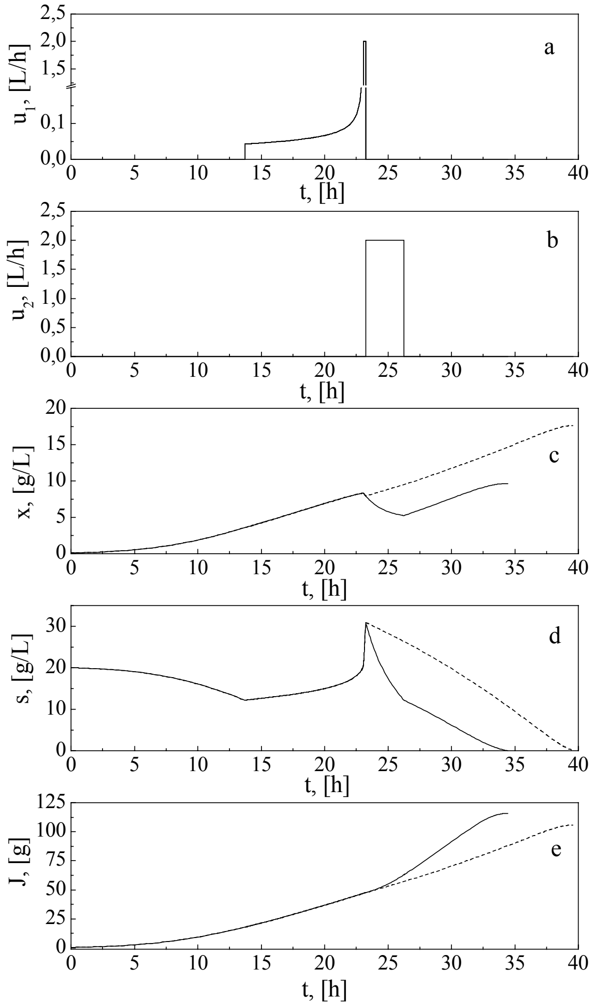

Figure 4. Results of process optimization for the fixed initial technological conditions calculated using the maximum principle based optimization algorithm (dotted lines present the process with the feed-rate control only)

Acknowledgements

The financial support from Lithuanian State Science and Studies Foundation (grant T-66/06) is gratefully acknowledged.

References

[1] **B.J. Minihane, D.E. Brown.** Fed-batch culture technology. *Biotechnology Advances*, *Pergamon Press*, 1986, *Vol*. 4, *No*.2, 2007-218.

[2] **H.C. Lim, K.S. Lee.** Control of bioreactor systems. *In: Biotechnology*, *Vol*.4, *Measurement, modeling and control, Schugerl, K. (Ed.), VCH, Weinheim*, 1996, 506-560.

[3] **J.H. Lee, H.C. Lim, S.J. Kim.** A nonsingular optimization approach to the feed rate profile optimization of fedbatch cultures. *Bioprocess and Biosystems Engineering*, 2001, *Vol*.24, 115-121.

[4] **D. Levišauskas, V. Galvanauskas, S. Henrich, K. Wilhelm, N. Volk, A. Luebbert.** Model-based optimization of viral capsid protein production in fed batch culture of recombinant Escherichia coli. *Bioprocess and Biosystems Engineering*, 2003, *Vol*.25, 255-261.

[5] **D. Sarkar, J.M. Modal.** Optimization of fed-batch bioreactors using genetic algorithm: multiple control variables. *Computers & Chemical Engineering*, 2004, *Vol*.28, *No*.5, 789-798.

[6] **D. Levišauskas, V. Galvanauskas, G. Žunda, S. Grigiškis.** Model-based optimization of biosurfatctant production in fed-batch culture *Azotobacter vinelandii*. *Biotechnology Letters*, 2004, *Vol*.26, *No*.14, 1141-1146.

[7] **V. Galvanauskas, N. Volk, R. Simutis, A. Lübbert.** Design of Recombinant Protein production Processes. *Chemical Engineering Communications*, 2004, *Vol*.191, *No*.5, 732-748.

[8] **H. Fukuda, T. Shiotani, W. Okada, H. Morikawa.** A mathematical model of the growth of bakers' yeast subject to production inhibition. *Ferment. Technol.*, *Vol*.56, 1978, 361-368.

[9] **J.C. Menezes, S.S. Alves, J.M. Lemos, S. Feyo de Azevedo.** Mathematical Modelling of Industrial Pilot-Plant Penicillin-G Fed-Batch Fermentations. *J. Chem. Tech. Biotechnol.*, *Vol*.61, 1994, 123-138.

[10] C.D. Johnson, J.E. Gibson. Singular solutions in problems of optimal control. *IEEE Transactions on Automatic Control*. *Vol*. AC-8, *No*.1, 1963, 4-15.

[11] W. Hager, P. Pardalos. (Eds.). Optimal Control: Theory, Algorithms and Applications. *Kluwer*, *Dodrecht*, 1998.

[12] E. Fraga, A. Žilinskas. Evaluation of hybrid optimization methods for the optimal design of heat integrated distillation sequences. *Advances in Engineering Software*, *Vol*.34, 2003, 73-86.

[13] A. Žilinskas, E. Fraga, A. Mackutė. Data analysis and visualisation for robust multi-criteria process optimization. *Computers & Chemical Engineering*, *Vol*.30, 2006, 1061-1071.

Received October 2006.