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OPTIMIZATION OF FEED-RATE IN FED-BATCH CULTURE *ENTEROBACTER AEROGENES* 17 E13 FOR MAXIMIZATION OF BIOMASS PRODUCTIVITY

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Abstract. The paper presents the results of model-based optimization of fed-batch culture *Enterobacter aerogenes* 17 E13 for maximization of biomass productivity. A mathematical model for the problem solving is developed using mass-balance conditions for cells' biomass and limiting substrate, relevant kinetic relationships for modeling the specific rates of biochemical transformations, and experimental data from fed-batch cultivation processes, realized under various cultivation conditions. The optimization procedure refers to the stochastic search algorithm based parametric optimization of flexible time function approximating the feed-rate time profile.

Keywords: mathematical model, optimization, fed-batch cultivation process.

1. Introduction

Fed-batch operating mode of bioreactors provides important advantage over the batch operation and is widely used in fermentation technology [1]. In fed-batch fermentation processes, concentration of substrate in cultivation medium can be externally controlled by manipulating the feed-rate. Therefore, the substrate concentration dependent state of microorganisms' culture that is optimal for biosynthesis of desired product can be maintained by using appropriate feed-rate profiles. Currently, the feed-rate optimization problems are commonly solved by mathematical model-based optimization methods.

During last few decades several optimization methods and algorithms have been developed for solving the feed-rate optimization problems. For simple mathematical models consisting of 3-4 nonlinear differential equations, the Maximum principle based optimization techniques can be applied [2–4]. Application of the optimization technique based on dynamic programming is presented in [5]. For complicate models that include higher number of state equations, and hybrid models that combine first principle models with artificial neural networks, the parametric optimization approach is the most attractive due to possibility to apply well-developed nonlinear programming and stochastic search algorithms [6, 7]. The latter approach of the feed-rate optimization is attractive also for the reason that it requires less effort to develop the calculation algorithm, as compared with the alternative approaches.

Feed-rate optimization problem presented in this paper is related to the development of complex technology for grease wastes utilization. Biopreparation for the grease wastes utilization is produced by cultivation of the microorganisms culture *Enterobacter aerogenes* 17 E13 in a bioreactor of fed-batch operating mode. Technological goal of the optimization is to maximize production of biomass at the end of fed-batch cycle. Solving of the optimization problem requires development of the process mathematical model. However, identification of mathematical model is complicated by a factor that concentrations of the substrate organic components are not directly measured.

In the present paper, the relevant mathematical model has been developed using biomass growth data and initial values of substrate concentrations of fed-batch cultivation experiments realized in flasks and laboratory-scale bioreactor. The optimization problem is solved using stochastic search algorithm applied for optimization of the of radial basis functions network approximating the feed-rate time profile.

2. Experimental setup

Experiments of fed-batch cultivation of *Enterobacter aerogenes* 17 E13 were carried out in flasks and laboratory bioreactor. The 750 ml Erlenmeyer flasks containing 70 ml of complex nutrient medium were incubated at 30 °C in a rotary shaker Innova 43 (New Brunswick Scientific Co.) at 200 rpm for 16 h. The 14 l laboratory bioreactor BioFlo 110 (New Brunswick Scientific Co.) is equipped with the systems for controlling the technological parameters at desired levels (temperature 30 °C, pH 6.5-7.5, aeration 4 -7 l/min and the agitation 200-400 rpm). The desired feed-rate time profile was realized as a program control algorithm via integrated computer-bioreactor system.

During cultivation experiments, the samples of cultural broth were taken at a sampling time of 1 h to estimate the bacteria cell concentration. The cell concentration, defined as colonies forming units per liter (*CFU* l^{-1}), was determined by plating diluted culture broth on nutrient agar (Oxoid) plates and incubating at 30 °C for 24 h.

Calculations related to the process model identification, model-based optimization and the simulation experiments were performed using Matlab/Simulink tools.

3. Development of mathematical model

The state model for solving the dynamic optimization problem is based on the mass balances for cell biomass, substrate and volume of cultural liquid:

$$\frac{dx}{dt} = \mu x - \frac{F_s}{V} x , \tag{1}$$

$$\frac{ds_i}{dt} = -q_{si} x - \frac{F_s}{V} s_i + \frac{F_s}{V} s_{i,f} , \quad i = 1, 2, 3 , \tag{2}$$

$$\frac{dV}{dt} = F_s - F_{samp} , \tag{3}$$

where x, s_1, s_2, s_3 are concentrations of biomass, sunflower oil, casein hydrolysate and yeast extract, respectively, in cultivation medium; $s_{1,f}$, $s_{2,f}$, $s_{3,f}$ are concentrations of the substrate components in the feeding solution, μ – specific rate of cells' biomass growth, q_{si} is specific consumption rate of the *i*-th substrate component, V is volume of cultivation medium; F_s is feed rate of the substrate solution (control variable), F_{samp} is sampling rate.

In order to identify relevant structure of functional relationship for μ in the model (1), several kinetic relationships for the specific rate of biomass growth [8, 9] have been investigated:

$$\mu(x, s_1) = \mu_{\max} \frac{s_1}{s_1 + x K_{xx}} , \tag{4}$$

$$\mu(x, s_1) = \mu_{\max} \frac{s_1}{s_1 + K_s} \frac{K_{xx}}{x + K_{xx}} , \tag{5}$$

$$\mu(x, s_1) = \mu_{\max} \frac{s_1}{s_1 + K_s} \frac{K_i}{s_1 + K_i} - K_{xx} x , \tag{6}$$

where $\mu_{\max}, K_s, K_i$, and K_{xx} are model parameters describing maximal specific growth rate, Monod constant, substrate inhibition constant, and biomass inhibition constant, respectively.

Modeling of the specific substrate consumption rate q_{si} refers to the assumption about linear relationship between the biomass growth and substrate consumption rates:

$$q_{s1} = \frac{1}{Y_1} \mu + m , \tag{7}$$

$$q_{s2} = \frac{1}{Y_2} \mu , \tag{8}$$

$$q_{s3} = \frac{1}{Y_3} \mu , \tag{9}$$

where Y_1, Y_2, Y_3 are yield coefficients, m is maintenance term.

Analysis of experimental data has shown that duration of the lag-phase at which adaptation of the microorganisms to the environment takes place is weighty compared with the overall process time. This phenomenon is described in the mathematical model by introducing an auxiliary factor $\alpha(t)$ in series with the functional relationship $\mu'(x, s)$.

$$\alpha(t) = \frac{arctg\left(K_p \left(\frac{t - t_0}{\tau} - 1 \right) \right)}{\pi} + 0.5 , \tag{10}$$

where t is process duration, K_p, t_0, and τ are model parameters subjected to identification.

The resultant specific growth rate $\mu(x, s)$ in the model equation (1) is determined by the following functional relationship:

$$\mu(x, s) = \mu'(x, s) \cdot \alpha(t) , \tag{11}$$

where the expression for $\mu'(x, s)$ finally is chosen from the equations (4)–(6) based on the model identification quality of each of the relationships.

Identification of the kinetic relationships (4)–(9) is complicated by the fact that the substrate (sunflower oil, casein hydrolysate and yeast extract) concentrations are not directly measured. Therefore, the parameters of the functional relationships are to be identified using the biomass growth data and initial values of substrate concentrations only.

In order to identify the mathematical model that is capable to predict the process behavior under various operating conditions, experimental data of several fed-batch biomass growth processes realized in flasks and laboratory were used in the identification procedure.

The procedure of identification the model parameters is implemented using numerical search algorithm based on evolutionary programming techniques that calculate the optimal values of parameters of the nonlinear dynamic model (eqs. (1)–(3), (6)–(11)). By the search procedure a vector of the model parameters $\mathbf{P} = [\mu_{\max}, K_s, K_i, K_{xx}, K_p, t_0, \tau, Y_1, m]$ is determined that minimizes the criteria of the model quality (root mean square error, [10]). The objective function for the identification procedure is the sum of weighted squared deviations between the state variables of the model (y_{ij}) and the corresponding experimental data ($y_{e,ij}$):

$$J(\mathbf{P}) = \sqrt{\frac{\sum_{j=1}^{k}\sum_{i=1}^{n} w_{ij}\left(y_{ij}(\mathbf{P}) - y_{e,ij}\right)^2}{kn}} \to min, \quad (12)$$

where w_{ij} – weight coefficients, i - index of the experiment, j – index of the measured variable, n – number of experiments, k – number of measured variables.

The values of the weight coefficients w_{ij} are chosen empirically taking into account the importance of particular variable for the identification task, the accuracy and reliability of a particular variable measurement, and the variation range of variables. Different importance of various experiments in the identification procedure is also evaluated using the weight coefficients.

The model parameters are identified using the global search stochastic algorithms (evolutionary programming [11]).

By comparison of the values of the objective function (12) calculated during identification of the models with different expressions (4)–(6) for modeling of the specific growth rate μ it is determined that the best approximation of experimental data is obtained with the functional relationship (6). The identified values of the model parameters are presented in Table 1. The identification results are presented in Figure 1.

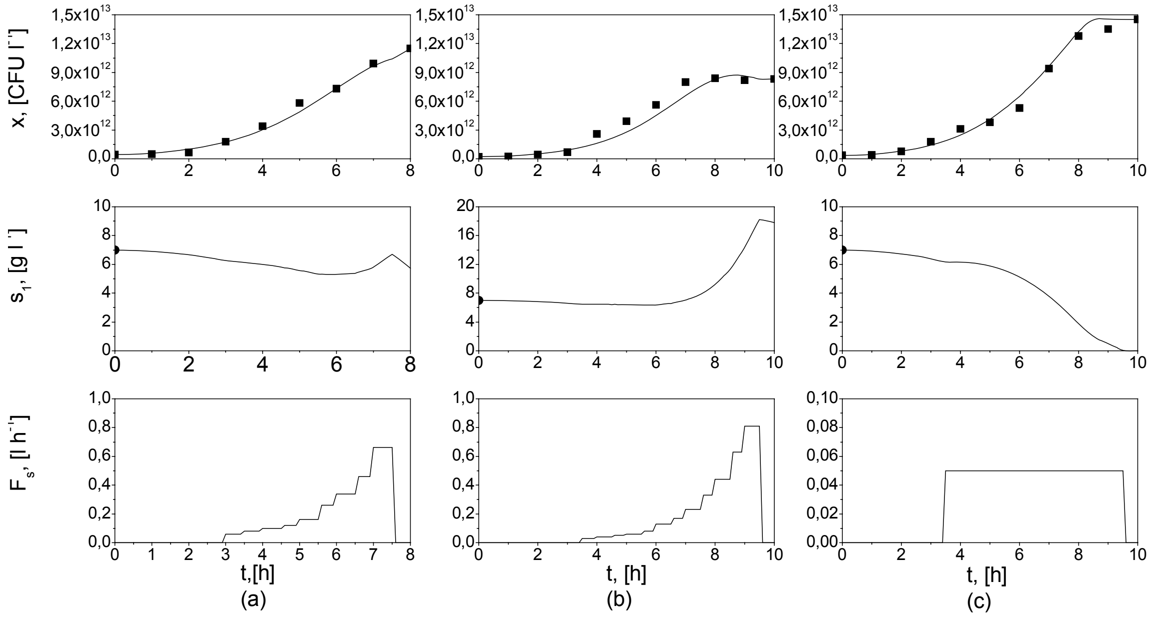

Figure 1. Approximation of experimental data (symbols) from fed-batch cultivation processes realized in flask (a) and laboratory bioreactor (b, c) by the mathematical model (1)–(3), (6)–(11) (lines) with the parameter values given in Table 1

Table 1. Model parameter values used in the simulation

Model parameter	Value	Units
$\mu_{\max}$	2.0	h^{-1}
K_s	1.31	$g\ l^{-1}$
K_i	4.08	$g\ l^{-1}$
K_{xx}	4.05×10^{-14}	$CFU^{-1}\ l\ h^{-1}$
K_p	1.20	–
τ	0.25	h
t_0	0.32	h
Y_1	2.83×10^{12}	$CFU\ g^{-1}$
Y_2	5.14×10^{12}	$CFU\ g^{-1}$
Y_3	4.23×10^{12}	$CFU\ g^{-1}$
m	1.14×10^{-13}	$g\ CFU\ h^{-1}$

4. Model-based feed-rate optimization

The goal of optimization is to maximize production of biomass at the end of fed-batch cultivation process of unspecified duration. The manipulated variable is feed-rate. Process duration is indirectly restricted by the fixed amount of substrate committed for the cultivation process.

The mathematical model applied for solving of the optimization problem consists of the set of state equations (1)–(3) and the functional relationships (6)–(11) described in the previous section. The model parameter values, initial values of the state variables and concentrations of the substrates in the feeding solution are given in Table 1 and Table 2, respectively.

The optimization problem is formulated as follows.

The objective function, as quantified by:

$$J = x(T)V(T) \rightarrow \max, \tag{13}$$

where T is duration of fed-batch cultivation process cycle, and has to be maximized by manipulating the feed-rate while satisfying restrictions on the feed pump allowed feed-rate variation interval

$$0 \le u \le u_{\max}, \tag{14}$$

the volume of cultural liquid

$$V \le V_{\max}, \tag{15}$$

and the total volume of substrate solution fed into bioreactor:

$$\int_0^T F_s dt \le V_{s,\max}, \tag{16}$$

where $u_{\max}$ is the maximum feed rate, $V_{\max}$ is the maximum permissible volume of cultural liquid in bioreactor and $V_{\mathrm{s,\,max}}$ is maximum permissible volume of substrate solution fed into bioreactor.

Table 2. Boundary values of the state variables and values of technological parameters used in the simulation

State variables and substrate concentrations in feeding solution	Value	Units
$x(0)$	5.8×10¹¹	CFU l^{-1}
$s_1(0)$	7.0	g l^{-1}
$s_2(0)$	9.0	g l^{-1}
$s_3(0)$	6.0	g l^{-1}
$V(0)$	7.0	1
$V_{\max}$	8.0	1
$V_{s,\,\max}$	0.7	1
$u_{\max}$	1.0	1 h^{-1}
$s_{1,f}$	100.0	g 1^{-1}
$s_{2,f}$	100.0	g 1^{-1}
$s_{3,f}$	100.0	g 1^{-1}

The above dynamic optimization problem is reduced by applying the parametric optimization approach to calculate the optimal feed-rate time profile. In previous investigations of this approach for solving the model-based feed-rate optimization problems [7] it was shown the that close-to-optimal feed-rate time-profiles can be calculated by approximating the optimal profiles by flexible time functions containing modest number of parameters and by applying the nonlinear programming or stochastic search algorithms for calculation of the function parameter values that correspond to the optimal feed-rate time profile.

In this paper, we applied a network of the radial basis functions for approximation the feed-rate time profile:

$$f(t) = \sum_{i=1}^{I} w_i \Phi_i, \tag{17}$$

where Φ_i are Gauss‘s functions describing bell shape relationships:

$$\Phi_i = exp\left(-\frac{r_i^2}{\rho_i^2 + \xi}\right), \tag{18}$$

$$r_i = t - c_i, \tag{19}$$

where ξ is a small constant introduced to ensure numerical stability; c_i, w_i, ρ_i are parameters subjected to optimization.

The transformed optimization problem is to determine the values of the approximating function (17) parameters, that determine optimal feed-rate time profile maximizing the performance index (13). In our application, the network of 10 functions (18) was used.

In order to speed up convergence of the iterative search algorithm, the first approach feed-rate time profile was calculated. The preliminary feed-rate was calculated to keep the substrate concentrations at the level that corresponds to the maximum specific growth rate determined from experimental data. The feed-rate providing for such technological conditions is calculated by solving the model equations (1)-(3), (6)-(11) in which

$$s_1 = s_{1,opt}, \tag{20}$$

$$ds_1/dt = 0, \tag{21}$$

$$F_{s,opt} = \frac{x(t)V(t)}{\left(s_{1,f} - s_{1,opt}\right)}\left(\frac{\mu_{\max}}{Y_1} + m\right), \tag{22}$$

where $s_{1,opt}$ is substrate (sunflower oil) concentration at which biomass specific growth rate gains its maximum value.

The first approach feed-rate profile was further approximated by the radial basis function network (17)–(19), and the calculated network parameter values were used as the first approach values in the foregoing iterative parametric optimization procedure. The optimized feed-rate time profile and time trajectories of the state variables and objective function are presented in Figure 2.

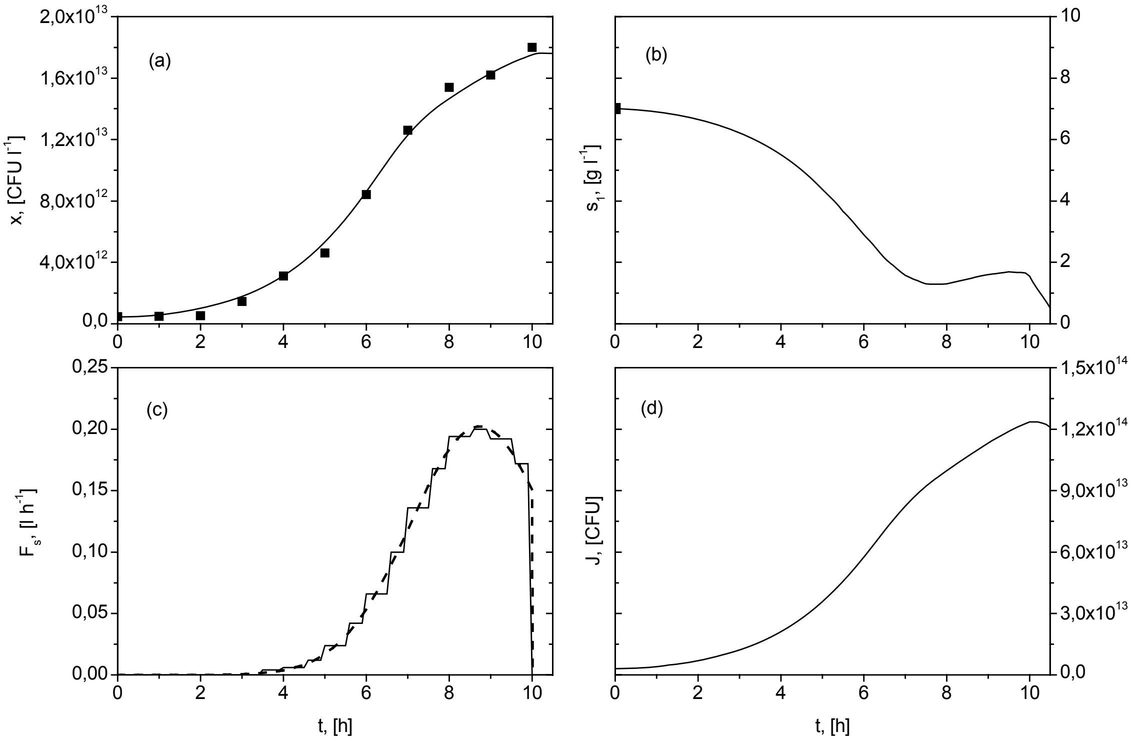

Figure 2. Simulated time trajectories of the optimized process variables (lines) and experimental data of the validation experiment (symbols)

The yield of cells' biomass at the end of optimal process (t = 10 h) totals 1.24×10^{14} CFU and is for 25 % higher as compared to the best result (0.98×10^{14} CFU) obtained in experiments that were used for identification of the process mathematical model. Experimental testing of the calculated feed-rate control algorithm validated the model-based optimization results. The feed-rate time profile in the validation experiment was realized as step-wise approximation of the calculated optimal program control algorithm as shown in Figure 2c. Doted line in Figure 2c depicts the output of radial basis function network.

The coincidence of the experimental data and the simulated biomass growth time trajectory of the optimal process is shown in Figure 2a.

5. Conclusions

The feed-rate for fed-batch cultivation of the microorganisms culture *Enterobacter aerogenes* 17 E13 is optimized in order to maximize the yield of cells' biomass at the end of fed-batch cycle.

Solving of the optimization problem refers to the mathematical model of fed-batch culture that is developed using the biomass growth and the substrate initial concentration data of batch and fed-batch cultivation experiments realized at various cultivation conditions.

The model-based optimization problem is solved in two steps. At the first step, the first approach feed-rate profile is calculated based on preliminary assumptions about technological conditions of the optimal process. At the second step, the first approach feed-rate time profile is optimized using parametric optimization procedure of the radial functions network approximating the feed-rate time profile. In the optimization procedure, the stochastic search (evolutionary programming) algorithm is applied.

According to the simulation results and the validation experiment of the optimized fed-batch cultivation process, increase of the biomass yield due to optimization totals over 25 % as compared to the best result obtained in experiments that were used for identification of mathematical model.

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