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# MODEL-BASED OPTIMIZATION OF FED-BATCH FERMENTATION PROCESSES USING PREDETERMINED TYPE FEED-RATE TIME PROFILES. A COMPARATIVE STUDY.

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**Abstract**. The paper presents the model-based feed-rate optimization results for various fed-batch fermentation processes obtained by using predetermined types of the feed-rate time profile approximating functions and the parametric optimization procedure. Optimization results obtained for various feed-rate time profiles are compared and practical recommendations for selection the structure of approximating function are given.

**Key words:** mathematical model, fed-batch fermentation process, optimization.

## 1. Indroduction

In fed-batch fermentation processes concentration of substrate in cultivation medium can be externally manipulated by altering the feed-rate. Therefore, an optimal state of microorganisms' culture for biosynthesis of the desired product can be maintained by using appropriate feed-rate profiles. Feed-rate optimization in fed-batch fermentation processes is one of widespread problems in biotechnology. Currently, the above optimization problem is commonly solved by mathematical model based optimization methods [1-5,7]. For simple mathematical models consisting of 3-4 nonlinear differential equations the Maximum principle based optimization techniques can be applied [2]. 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 random search algorithms [3,5,7].

By applying the parametric optimization approach a reasonable time function for approximation of the optimal feed-rate time profile is to be selected *a priori* and optimal parameter values of the approximating function are to be calculated. An extent of calculations and accuracy of optimal solution depend on a number of parameters of the approximating function subjected to optimization. The number of the time function parameters can vary from 3 in a steady value function to 10-30 in a network of radial basis functions.

The aim of this work is to investigate an impact of the approximating function structure on the process optimization results and to formulate recommendations for the structure selection in order to achieve a compromise between accuracy of solution and extent of calculations. The investigation is carried out using mathematical models developed for solving optimization problems of various fermentation processes [4-7].

## 2. Time functions for the feed-rate time profile optimization

A typical fed-batch fermentation process consists of three technological stages: batch-feeding-batch. Optimization problem is to determine the feed start and finish time points and the feed-rate time profile during the feeding time interval. An optimal feed-rate time profile is usually close to exponential, however, the simplified time profiles such as a constant rate or a ramp shape profiles can give process optimization results close to optimal.

In this paper, we have investigated 4 types of time functions approximating the optimal feed-rate time profile in the model-based optimization procedure.

The 1st type is constant feed-rate:

$$u(t) = a \qquad (1)$$

and an objective of optimization is to calculate the parameter $a$ along with the feed start ($t_{st}$) and the feed end ($t_{end}$) time points that maximize performance index of a particular fed-batch fermentation process.

The 2nd type is a ramp-type function:

$$u(t) = a + bt \qquad (2)$$

and parameters to be optimized are *a, b,* $t_{st}$, $t_{end}$. The 3rd type is an exponential function of the following structure:

$$u(t) = a\,exp(b + ct) \quad (3)$$

with the parameters *a, b,c,* $t_{st}$, $t_{end}$ to be optimized.

And the 4th type is a network of radial basis functions that can precisely approximate complex time profiles:

$$u(t) = \sum_{i=1}^{5} w_i \Phi_i(t) \ , \quad (4)$$

$$\Phi(t) = exp\left(-\frac{(t\text{-}d_i)^2}{\rho_i^2}\right), \quad (5)$$

where $w_i$ are weighting coefficients, $d_i$, $\rho_i$ are parameters of bell-shape functions. An optimal time profile of the feed-rate is obtained by optimization of the above parameter values.

For solving the parametric optimization problems we applied *chemotaxis* random search algorithm [8].

## 3. Examples of the predetermined type feed-rate time profiles optimization for various fed-batch bioprocesses

*Optimization of E. coli cultivation process for biomass production*

The mathematical model is developed in [4] and has the following form:

$$\frac{dX}{dt} = \mu X - u\frac{X}{V} \ , \quad (6)$$

$$\frac{dS}{dt} = -q_S X + u\frac{S_F - S}{V} \ , \quad (7)$$

$$\frac{dV}{dt} = u \ , \quad (8)$$

with the boundary conditions:

$X(0) = 1.0$ opt.units, $S(0) = 16.7$ g/L, $V(0) = 5.0$ L, $V(T) = 5.9$ L,

where *X* and *S* are concentrations of biomass and substrate, respectively; $S_F$ is feed substrate concentration ($S_F = 200$ g/L); *V* is a volume of fermentation broth; *u* is the feed rate; $\mu$, $\rho_s$ are specific rates of cell growth and substrate consumption, respectively; *t* is a time; *T* is a duration of the fermentation process.

The restriction is imposed on the feed rate

$$0 \le u \le 1.5 \text{ L/h}. \quad (9)$$

Specific rates of cell growth and substrate consumption are described by the following expressions:

$$\mu = 0.0713 \cdot S - 0.00690 \cdot X \ , \quad (10)$$

$$q_S = 0.157 - 0.0107 \cdot S + 0.00163 \cdot S^2 \ . \quad (11)$$

An objective of optimization is to find the feed-rate profile that maximizes yield of cells biomass at the end of cultivation process

$$J = X(T)V(T) \to \max \ , \quad (12)$$

where *T* is unfixed process duration.

Calculation with the PC (Pentium 1,7GHz) of the optimal feed-rate control algorithms using the time functions (1)-(4) discussed in the previous section took 2 min, 4 min, 6 min and 7.5 min, respectively.

The calculated optimal feed-rate time profiles and the corresponding yields of biomass are given in Figure 1.

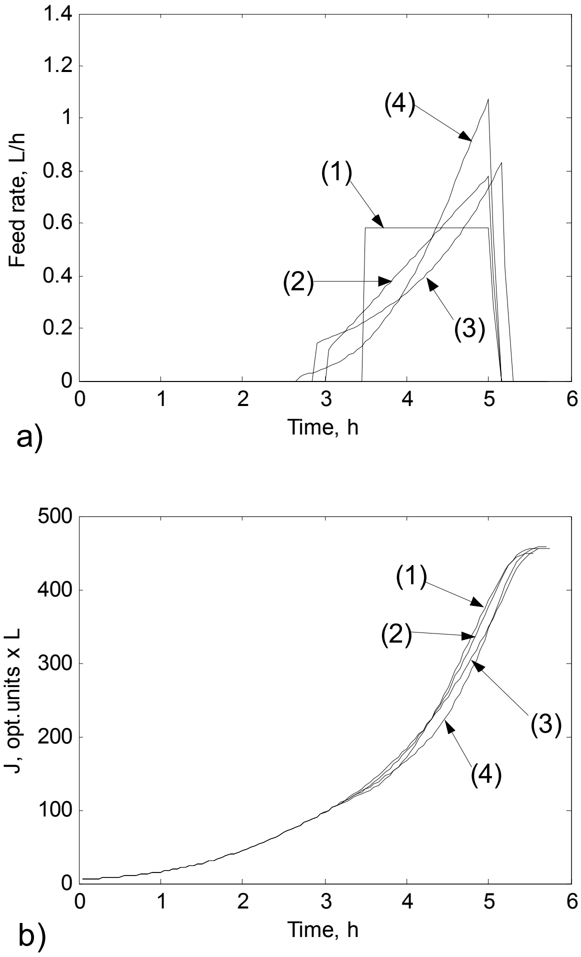



**Figure 1.** Optimization of *E.coli* cultivation process for biomass production using various feed-rate time profiles:
a – feed-rate time profiles,
b – time trajectories of biomass accumulation,
(1) - (4) – number of equation

The difference between the all calculated yields does not exceed 1.8 % from the maximum value, obtained with the feed-rate time profile approximated by the network of radial basis functions (1.8% using the time function (1), 0.5% using the time function (2), and 0.3% using the time function (3) ).

*Optimization of penicillin G production process by Saccharomyces cerevisiae*

The mathematical model is developed in [9] and has the following form:

$$\frac{dX}{dt} = \mu X - u\frac{X}{V} \ , \qquad (13)$$

$$\frac{dS}{dt} = -q_S X + u\frac{S_F - S}{V} \ , \qquad (14)$$

$$\frac{dP}{dt} = q_P X - u\frac{P}{V} \ , \qquad (15)$$

$$\frac{dV}{dt} = u \ , \qquad (16)$$

with the boundary conditions:

$X(0) = 1.29$ g/L, $S(0) = 69.02$ g/L, $P(0) = 0.0$ g/L, $V(0) = 8.121$ L, $V(T) = 10.0$ L,

where *X, S* and *P* are concentrations of biomass, substrate and product, respectively; $S_F$ is feed substrate concentration ($S_F = 500$ g/L); *V* is a volume of fermentation broth; *u* is the feed rate; $\mu$, $q_S$ and $q_P$ are specific rates of cell growth, substrate consumption and product formation, respectively; and *t* is a time.

The restriction is imposed on the feed rate

$$0 \le u \le u_{max} = 0.1 \text{ L/h}. \qquad (17)$$

Specific rates of cell growth, substrate consumption and product formation are described by the following functional relationships:

$$\mu = \frac{0.11 \cdot S}{0.006X + S} \qquad (18)$$

$$q_S = \frac{1}{0.47}\frac{0.11 \cdot S}{0.06X + S} + \frac{1}{1.2}\frac{0.0055 \cdot S}{0.0001 + S + \frac{S^2}{0.1}} + 0.029 \qquad (19)$$

$$q_P = \frac{0.0055 \cdot S}{0.0001 + S + \frac{S^2}{0.1}} - 0.01\frac{P}{X} \qquad (20)$$

The objective function is

$$J = P(T)V(T) \to \max , \qquad (21)$$

where *T* is unspecified process duration.

Calculation with the PC of the optimal feed-rate control algorithms using the time functions (1)-(4) took 4.3 min, 13.4 min, 23 min and 49 min , respectively.

The calculated optimal feed-rate time profiles and the corresponding amount of product are given in Figure 2.

The difference between the calculated yields of product does not exceed 0.3 % from the maximum value, obtained with the feed-rate time profile approximated by the network of radial basis functions (0.3% using time function (1), 0.06% using time functions (2) and (3)).

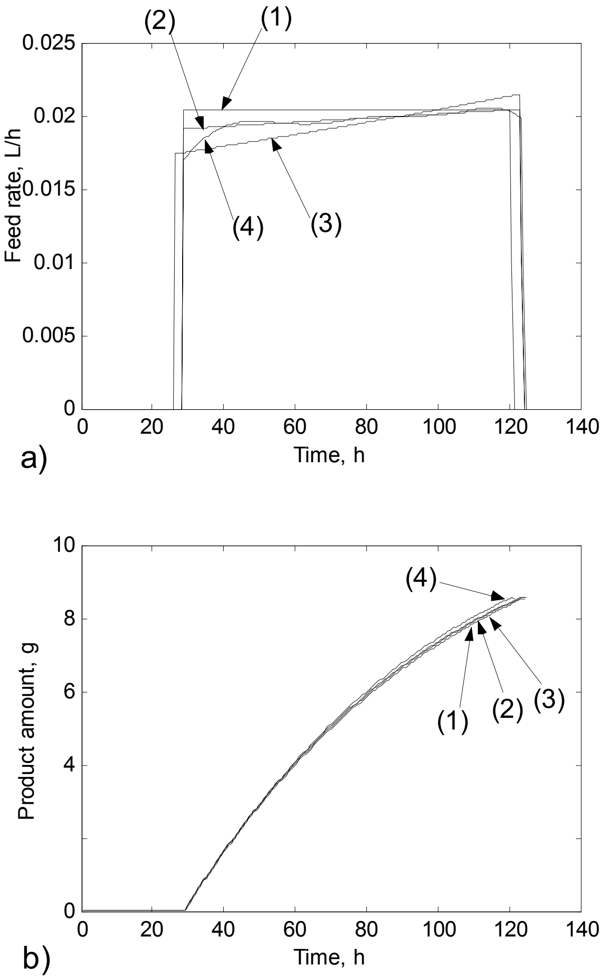



**Figure 2.** Optimization of the penicillin G production process by *Saccharomyces cerevisiae* using various feed-rate time profiles: a – feed-rate time profiles, b – time trajectories of product accumulation, (1)-(4) – number of equation

*Optimization of Azotobacter vinelandii cultivation process for biosurfactant production*

For solving of the optimization problem mathematical model of the following structure is developed [10] :

$$\frac{dX}{dt} = \mu X - u\frac{X}{V} \ , \qquad (22)$$

$$\frac{dS_1}{dt} = -q_{S1} X + u\frac{S_{1F} - S_1}{V} \ , \qquad (23)$$

$$\frac{dS_2}{dt} = -q_{S2} X + u\frac{S_{2F} - S_2}{V} \ , \qquad (24)$$

$$\frac{dS_3}{dt} = -q_{S3} X + u\frac{S_{3F} - S_3}{V} \ , \qquad (25)$$

$$\frac{dP}{dt} = q_P X - u\frac{P}{V} \ , \qquad (26)$$

$$\frac{dV}{dt} = u \ , \qquad (27)$$

with the boundary conditions:

$X(0) = 1.4$ g/L , $S_1(0) = 22.5$ g/L ,

$S_2(0) = 0.616$ g/L, $S_3(0) = 0.0292$ g/L,

$P(0) = 0.08$ opt.units, $V(0) = 3$ L ,

where $X$ and $P$ are concentrations of biomass and biosurfactants, respectively; $S_i$ are concentrations of considered substrate components ($S_1$ – glucose, $S_2$ – ammonia nitrogen and $S_3$ – phosphate phosphorus); $V$ is volume of cultural liquid in bioreactor; $u$ is feed rate; $S_{1F}$, $S_{2F}$ and $S_{3F}$ are substrate component concentrations in feed solution ($S_{1F}$ = 200 g/L, $S_{2F}$ = 3.42 g/L and $S_{3F}$ = 1.14 g/L).

$\mu$ is specific rate of biomass growth:

$$\mu = 1.388 \frac{S_1}{3.2337 \cdot X + S_1 + \frac{S_1^2}{24.1029}} - 0.0219 \cdot X \quad , \qquad (28)$$

$q_{Si}$ are specific rates of substrate components consumption:

$$q_{S1} = 0.1492 \cdot \mu \quad , \qquad (29)$$

$$q_{S2} = 0.0032 + 0.7898 \cdot 10^{-4} \cdot \mu + 0.2164 \cdot 10^{-4} \cdot q_P \quad , \qquad (30)$$

$$q_{S3} = 0.0012 + 4.0789 \cdot 10^{-3} \cdot \mu \quad , \qquad (31)$$

$q_p$ is specific rate of biosurfactants production. In this model, $q_p$ is estimated by artificial neural network (ANN), containing 6 inputs, 3 nodes in hidden layer and 1 output. Inputs of the ANN are current concentrations of glucose, ammonia nitrogen and phosphate phosphorus, and discrete measurements of biomass concentration from moving window of length 3 h.

The objective of the process optimization is to maximize the yield of biosurfactants at the end of fed-batch cycle by manipulating the feed rate. The performance index is

$$J = P(T)V(T) \to \max , \qquad (32)$$

where $T$ is the unspecified process duration.

The constraints are imposed on the feed rate:

$$0 \le u \le u_{\max} = 0.5 \text{ L/h} \qquad (33)$$

and the process duration:

$$T \le 11 \text{ h} . \qquad (34)$$

Calculation with the PC of the optimal feed-rate control algorithms using the time functions (1)-(4) took 4.3 min, 8.5 min, 20 min and 21 min , respectively.

The calculated optimal feed-rate time profiles and the corresponding yields of biosurfactants are given in Figure 3.

The difference between the calculated amounts of product does not exceed 4.9% from the maximum value, obtained with the feed-rate time profile approximated by the network of radial functions (4.9% using the time function (1), 2.0% using the time function (2), and 2.3% using the time function (3) ).

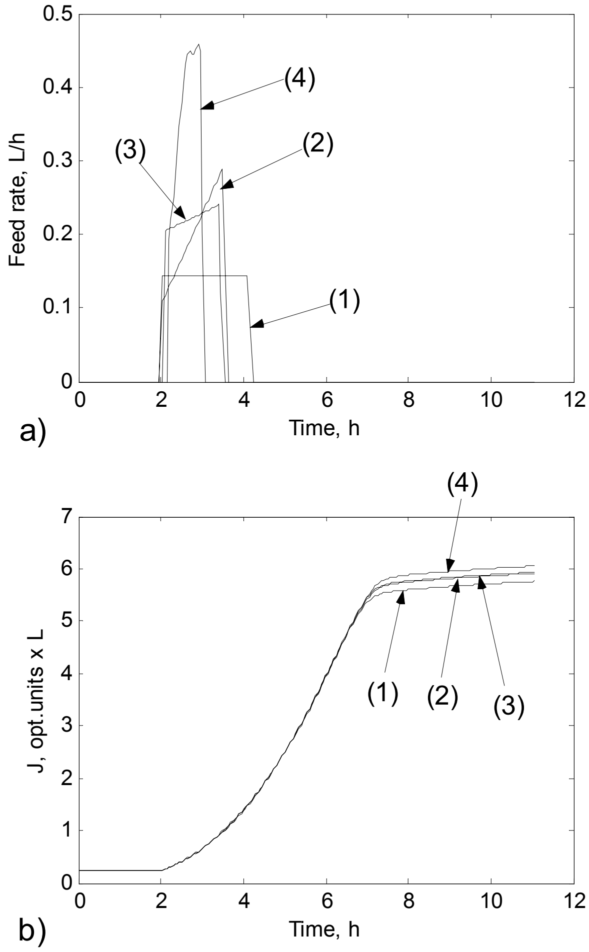



**Figure 3.** Optimization of *Azotobacter vinelandii* cultivation process for biosurfactant production using various feed-rate time profiles:
a – feed-rate time profiles,
b – time trajectories of product accumulation,
(1)-(4) – number of equation

*Optimization of E. coli cultivation process for recombinant protein production*

The mathematical model is developed in [5] and has the following structure:

$$\frac{dX}{dt} = \mu X - u\frac{X}{V} \quad , \qquad (35)$$

$$\frac{dAc}{dt} = -q_{Acc} X - u\frac{Ac}{V} \quad , \qquad (36)$$

$$\frac{dL}{dt} = -q_L X + u\frac{L_F - L}{V} \quad , \qquad (37)$$

$$\frac{dP}{dt} = q_p X - u\frac{P}{V} \quad , \qquad (38)$$

$$\frac{dV}{dt} = u \quad , \qquad (39)$$

with the boundary conditions:

$t \ge t_0 = 6$ h, $X(t_0) = 1.2$ g/kg, $Ac(t_0) = 0.8$ g/kg,

$L(t_0) = 32.0$ g/kg, $P(t_0) = 0.0$ g/kg, $V(t_0) = 4.5$ kg,

where $X$, $A_C$, $L$, $P$ are the concentrations of biomass, acetate, lactose and product, respectively. $L_F$ is concentration of lactose in feed ( $L_F = 210$ g/kg ). $V$ is the culture weight, $\mu$ is the specific growth rate of the biomass:

$$\mu = \frac{q_L}{2.2} \quad , \tag{40}$$

$q_{Acc}$ is the specific acetate consumption rate:

$$q_{Acc} = 2.15\frac{\text{Ac}}{\text{Ac}+0.11} \quad , \tag{41}$$

$q_L$ is the specific lactose consumption rate:

$$q_L = 1.74\frac{L}{L+0.77}\frac{0.001}{0.001+Ac}\frac{41.7}{41.7+L} \quad , \tag{42}$$

$q_P$ is the specific product biosynthesis rate:

$$q_P = \frac{q_L}{21.3}\frac{1}{1+\left(\frac{1.03}{L}\right)^2}\frac{1}{1+\left(\frac{0.50}{\mu}\right)^5} \quad . \tag{43}$$

The maximal feed-rate of lactose:

$$0 \le u \le u_{\max} = 0.8 \text{ kg/h.} \tag{44}$$

The maximum weight of culture liquid:

$$V \le V_{\max} = 6.0 \text{ kg.} \tag{45}$$

The restriction on the final cultivation time:

$$T \le 15.5 \text{ h} \tag{46}$$

The objective of the optimization is to determine the feeding time profile for lactose, which maximizes the performance index

$$J = P(T)V(T) \rightarrow \max \quad , \tag{47}$$

i.e., the amount of the product at the end of cultivation process.

Calculation with the PC of the optimal feed-rate control algorithms using the time functions (1)-(4) took 6.8 min, 12 min, 15 min and 23 min, respectively.

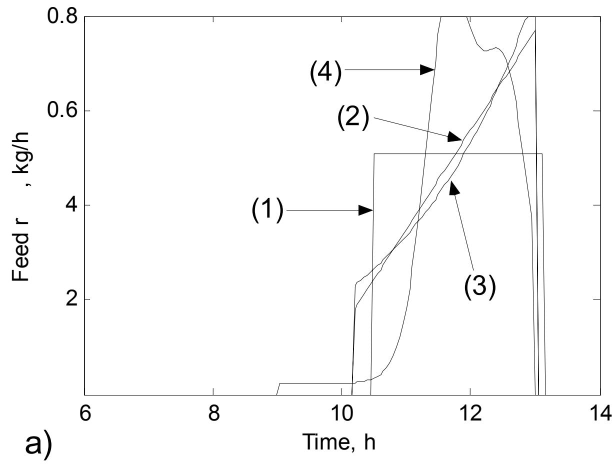
The calculated optimal feed-rate time profiles and corresponding amounts of the product are given in Figure 4.

The difference between the all calculated amounts of product does not exceed 7.5% from the maximum value, obtained with the feed-rate time profile approximated by the network of radial functions (7.5% using the time function (1), 2.9% using the time function (2), and 3.2% using the time function (3) ) .

## 4. Conclusions

In this study, practical aspects of the model-based fed-batch fermentation process optimization problem have been investigated. Investigation of the influence of the control action (feed-rate time-profile) shape on the process optimization results is carried out for various optimization problems: biomass, penicillin, biosurfactant and recombinant protein production.

The optimization results demonstrate that predetermined shape of the feed-rate time-profile (constant, ramp, exponential and complex (network of radial basis functions)) does not influence critically optimization results, which are closer related to an amount of the feed substrate. The differences between the calculated performance indices do not exceed 1.8 %, 0.3 %, 4.9 % and 7.5%, respectively, from the maximum value, obtained with the feed-rate approximated by the network of radial basis functions. An extent of calculations related to the optimization problem solving with the feed-rate approximating functions (2)-(4) took approximately 2, 4 and 8 times more iterations, respectively, as compared with the constant rate function (1).



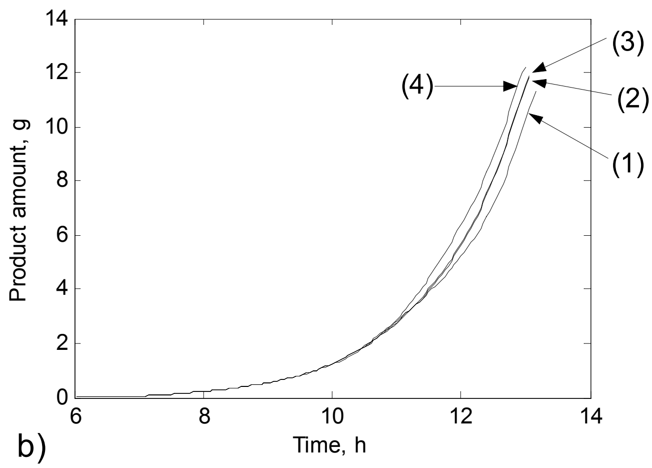




**Figure 4.** Optimization of *E. coli* cultivation process for recombinant protein production using various feed-rate time profiles: a – feed-rate time profiles, b – time trajectories of product accumulation, (1)-(4) – number of equation

Taking into account an extent of calculations related to optimization of the feed-rate time- profile approximating function parameters, the ramp-shape time function gives the best compromise between extent of calculations and optimization results: the mean difference from the best optimization result obtained with the network of radial basis functions was less than 1.4 % in all calculation experiments, and the calculation time was about 4 times shorter.

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