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Abstract: *This investigation deals with optimising the glucose feeding of the fed-batch process for the intracellular enzyme superoxide dismutase production on the basis of a hybrid model of the process. The control action has been the concentration of feeding glucose. The theoretical problem statement has been examined. The optimal profiles for glucose feeding of the process have been developed using three optimisation criteria.*

Keywords: *Enzyme SOD, Optimisation, Feeding profiles.*

Introduction

Superoxide dismutases (SODs) are metalloenzymes containing Mn, Fe or Cu/Zn in the active site that catalyze the disproportionation of superoxide free radical (O_2^-) to form hydrogen peroxide and dioxygen [1]. They have been considered as the first defence system against the cytotoxic superoxide free radical [2]. The main interest in these enzymes is due to their therapeutic potential. The use of SOD as a therapeutic agent has been proposed for several diseases □tumors, ischemic disease, arthritis, brain injuries, influenza, virus infections, etc. [1].

The production of SODs as intracellular enzymes determines its complicated nonlinear dynamics and difficulties with modelling and optimisation. There are almost no mathematical models of this process in the literature. In this study, the hybrid model described in [3] was used.

This paper deals with optimisation of the glucose feeding of the fed-batch process for SOD production on the basis of a hybrid model of the process.

Materials and methods

The fungal strain *H. lutea* 103 from the Mycological Collection of the Stephan Angeloff Institute of Microbiology at the Bulgarian Academy of Sciences □ Sofia, was selected as especially suitable for the biosynthesis of Cu/Zn-containing SOD. It was demonstrated to have a protective effect against myeloid Graffi tumor in hamsters and experimental influenza virus infection in mice [1].

Cultivation was performed in a 12 l bioreactor equipped with a pH-monitoring system, an automatic DO-monitoring and controlling system. The composition of the culture medium was described in [2]. The cultures were grown at 30°C for 72 h. The fermentation was at an impeller speed of 500 rpm and an air flow of 1 vvm.

The SOD activity was measured by the method of Beauchamp and Fridovich [4]. The protein was estimated by the Lowry procedure [5]. The soluble reducing sugars were determined by

the Somogyi-Nelson method [6]. The dry weight determination was performed on samples of mycelia harvested throughout the cultivation. The culture fluid was filtered off. The separated mycelia were washed twice with distilled water and dried to a fixed weight at 105°C.

For the fed-batch process, glucose was added at an interval of 3 h, starting in the 12th hour from the inoculation, in concentrations of 0.75, 1.0, 1.5, 2.0 and 2.5 g·l^{-1}. In the batch cultivation, cultures without glucose addition were used as a basis.

Results and discussion

Problem statement

Consider the class of bed-batch fermentation processes described by the model (1)-(4) with single limiting substrate [7, 8]:

$$\frac{dX}{dt} = \mu X - \frac{F}{V}X \tag{1}$$

$$\frac{dS}{dt} = -\rho X + \frac{F}{V}S_{0i} - \frac{F}{V}S \tag{2}$$

$$\frac{dP}{dt} = \xi X - \frac{F}{V}P \tag{3}$$

$$\frac{dV}{dt} = F \tag{4}$$

where: X is the biomass concentration, [g·l^{-1}]; S is the concentration of the limiting substrate, [g·l^{-1}]; P is the concentration of the objective product (in appropriate dimension); μ is the specific growth rate of the microorganisms, [h^{-1}]; ρ is the specific consuming rate of the limiting substrate, [h^{-1}]; ξ is the specific producing rate of the objective product, [h^{-1}]; V is the volume of cultural liquid in the bioreactor, [l]; F is the feeding rate, [h^{-1}]; S_{0i} is the concentration of the limiting substrate in the feeding solution, [g·l^{-1}].

If adding very small quantities (respectively taking out with the sampling) compared to the total volume V (this is compensated by big values of S_{0i}), F/V is very small. X and S are small also, so the last terms of Eqs. (1) and (2) can drop out.

The volume of the bioreactor V is always limited by some maximum value $V_{\max}$. There is always some initial quantity of the culture medium V_0. The following physical restriction could be written:

$$V(0) < V(t) < V_{\max} \tag{5}$$

In real working conditions during the process, sometimes from the bioreactor some quantities are pulled out for analyzing, so a more correct record of Eq. (4) is:

$$\frac{dV}{dt} = F_{\text{in}} - F_{\text{out}} \tag{6}$$

where: F_{in} is the flow rate of the input solution, F_{out} is the flow rate of a sample. If in the working time the condition $F_{\text{in}} = F_{\text{out}} = const$ is ensured, it follows from (6) that the volume of the bioreactor will be constant and Eq. (4) can drop out.

The control inputs can be assumed to be *F/V* or S_{0i}. In the model (1) □(4), there is only one possibility □to assume S_{0i} as control input, so the control enters linearly only the equation for *S*.

The statement of the optimal control problem is to define:

- the optimal initial condition *S**(0);
- the optimal feeding profile *u**(*t*) which minimizes the chosen cost function (CF).

According to [8], since the boundary conditions of the state vector and its costate vector (λ) are specified either at $t = 0$ or at $t = t_f$, this is a *two point boundary value problem* (*TPBVP*). This is our case. In a real life application, there is a constraint on the feeding pump capacity *u*(*t*):

$$0 = U_{\min} \le u(t) \le U_{\max} \tag{7}$$

where $U_{\max}$ is given.

An extremal control $u^*(t)$ can be determined by the optimal control sequence [$U_{\max}$, $U_{\min}$, u_{sing}] and the corresponding switching times.

In real conditions x_0 is known: *V*(0) is calculated, *S*(0) is fixed. Obviously, the optimal control sequence depends on *S*(0). It is also assumable that *X*(0) is low.

The realistic case for us is with high initial substrate concentration *S*(0). The growth phase is a complete batch phase, until at time $t = t_1$ *S* becomes small enough and *X* □high enough, S_0 that singular feeding can start.

Determining the singular control is very difficult even in the presence of reliable deterministic model, because the recognition of both the state and costate vectors of the system is necessary. The singular control profiles, known from similar tasks, are as shown on Fig. 1, starting from the moment t_1. In our investigations, one more new step was added, in which different u_{sing} levels are chosen for simulation, as shown on Fig. 1.

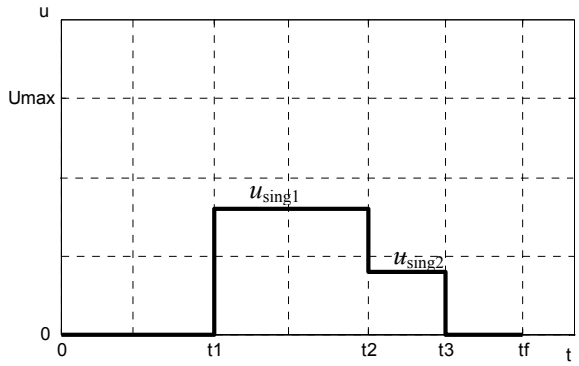

Fig. 1 Possible feed rate profile for high *S*(0)

Optimisation of the fed-batch process for the intracellular enzyme superoxide dismutase production with the control action being the concentration of feeding glucose

After analyzing and based on the generalized in [8] CFs, the following CFs (criteria) have been adopted:

1. $J_1 = -\frac{P}{t} \rightarrow min$ (8)

2. $J_2 = -\frac{P}{t + k_1 \int_0^t S_{0i} dt} \rightarrow min$ (9)

3. $J_3 = -P + k_2 t + k_3 \int_0^t S_{0i} dt \rightarrow min$ (10)

where P is the enzyme activity, t is the time for running the process, S_{0i} is the concentration of the feeding glucose and k_i $(i = 1 \div 3)$ are coefficients for dimensions. The first criterion reflects the enzyme production for minimum time, while in the second and third the economic side of the process is included also, i.e. besides maximum enzyme for minimum time, minimum expenses for feeding the process with glucose are sought.

For determining the optimal feeding profile, nonlinear constrained optimisation was used with the Optimisation toolbox for MATLAB and its gradient method Sequential quadratic programming. The optimisation procedure finds the minimum of a constrained nonlinear multivariable function. The previously obtained (by simulations) bounds for the main variables were used in the optimisation procedure, avoiding the estimations out of the admissible range and/or without physical sense.

The investigations with the hybrid model were done. The basic structure of the model is deterministic with the structure (1) □ (3) discussed above, but with the specific rates (of biomass growth, substrate consumption and enzyme production) modelled as neural networks [3].

Two types of input actions were used: constant S_{0i} and S_{0i}, changing on the previously defined profile.

Constant input action

The interval from 1 to 2 g·l^{-1} feeding glucose concentration was investigated for the constant S_{0i}, because this is the interval with increasing enzyme activity for increasing glucose feeding. For higher S_{0i} values, inhibition of the process was observed. The results for the enzyme activity and the CFs values are presented in Table 1, and the CFs curves □on Fig. 2. It is evident that according to all criteria the process is best with 2 g·l^{-1} glucose feeding, but it is advisable the process to be stopped earlier. Since the minimums of J_1 and J_2 are at the 29th and 28th hour, if the purpose is maximum enzyme for minimum time, the process could be stopped at the 29th hour. But then the enzyme is still very low. If the enzyme production predominates as a factor, then stopping the process at the 44th hour, where the enzyme is maximum, is advisable. As a compromise, we offer stopping the process at the minimum of J_3 at the 40th hour.

Input action with profile

According to the above presented theoretical investigations, it is necessary to define the following indexes (Fig. 1):

- time t_1
- feeding u_{sing}
- time t_3
- time t_f

Determining the time t_1 was done heuristically, using multiple sets of experimental data. It was determined to be at the 12th hour from the beginning of the process, since up to this moment the concentration of the glucose inside the bioreactor was decreased but not exhausted. Therefore, all the experiments were done with feeding starting at that moment.

Table 1

S_{0i} [g·l^{-1}] / t [h]	1	1.25	1.5	1.75	2.0
P_{max}	1770	1957.9	2215.6	2615.3	3666.5
t_{Pmax}	31	32	34	36	44
J_{1min}	-71.1	-74.4	-79.4	-87	-101.5
t_{J1min}	20	22	23	25	29
J_{2min}	-136.7	-141.2	-148.1	-159.5	-181.7
t_{J2min}	20	21	23	25	28
J_{3min}	-1313.8	-1432.3	-1606.3	-1895.9	-2664.5
t_{J3min}	28	29	31	33	40

Different profiles for glucose feeding were investigated. The process is provisionally separated into three phases:

1. From the 0th to the 12th hour of the process. During that phase glucose is not exhausted enough and feeding is not necessary. This is the interval [0, t_1] from Fig. 1.
2. Feeding phase from the 12th hour up to the moment t_3, when feeding can be stopped. This is the interval [t_1, t_3] from Fig. 1. This phase is characterised with intensive enzyme formation, while glucose is already exhausted, so feeding is necessary. Different profiles for different intervals were investigated. For completeness, the interval was divided into two subintervals: [t_1, t_2] and [t_2, t_3]. In each of them the feeding is different.
3. From t_3 to the end of the process. Corresponds to the interval [t_3, t_f] form Fig. 1. The optimisation investigations show that the feeding can be stopped before the end of the process without reflecting the criteria.

The results for the enzyme activity and the CFs values for some more important feeding profiles are shown in Table 2 and Table 3, and graphically on Fig. 3. In all the three criteria, the best results were obtained for the following feeding profiles: 0/2/1/0 g·l^{-1} and 0/2/0 g·l^{-1}. J_1 and J_2 reach their minimums at the 29th and the 28th hours when the enzyme is still very low. According to J_3, the best feeding profile is 0/2/0 (starting the feeding at the 12th hour and stopping it at the 36th hour) and the process should be stopped at the 40th hour, when the minimum of the criterion is reached. It is evident that the minimums of the three criteria are very close to one another. The criteria are shown on Fig. 4. The corresponding enzyme activities are shown on Fig. 5. As a result of the optimisation, the best feeding profile according to all criteria (high enzyme production for minimum expenses) is J_3. It is shown on Fig. 4 with continuous line. Following this criterion, the process time decreases with 45%, which leads to high economies of expenses, keeping the enzyme activity almost the same.

Table 2

S_{0i} [g·l^{-1}]	0/1/0	0/1/2/0	0/1/2/0	0/1/2/0	0/2/1/0
t [h]	0-12/12-67/67-72	0-12/12-30/30-58/58-72	0-12/12-20/20-57/57-72	0-12/12-15/15-51/51-72	0-12/12-15/15-68/68-72
P_{max}	1770	1770.1	2014.3	2357.6	2174
t_{Pmax}	31	32	36	36	33
J_{1min}	-71.1	-71.1	-71.1	-79	-81.5
t_{J1min}	20	20	20	25	22
J_{2min}	-136.7	-136.7	-136.7	-145.1	-153.9
t_{J2min}	20	20	20	24	22
J_{3min}	-1313.8	-1313.8	-1353.0	-1633.8	-1653.2
t_{J3min}	28	28	30	32	30

Table 3

S_{0i} [g·l^{-1}]	0/2/1/0	0/2/1/0	0/2/1/0	0/2/0	0/2/0
t [h]	0-12/12-20/20-64/64-72	0-12/12-25/25-55/55-72	0-12/12-35/35-45/45-72	0-12/12-36/36-72	0-12/12-41/41-72
P_{max}	2659.9	3090.9	3574.6	3594.2	3656.3
t_{Pmax}	33	36	41	40	43
J_{1min}	-94	-100.3	-101.5	-101.5	-101.6
t_{J1min}	25	27	29	29	29
J_{2min}	-173.4	-181.7	-181.8	-181.7	-181.7
t_{J2min}	25	27	28	28	28
J_{3min}	-2061.7	-2393.0	-2675.5	-2695.8	-2664.5
t_{J3min}	32	34	39	40	40

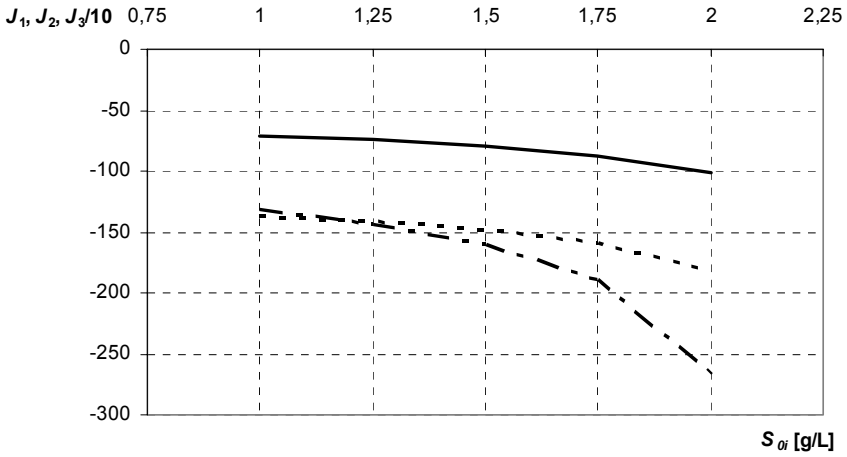

Fig. 2 J_1 ——, J_2 - - - -, $J_3/10$ - · - · -

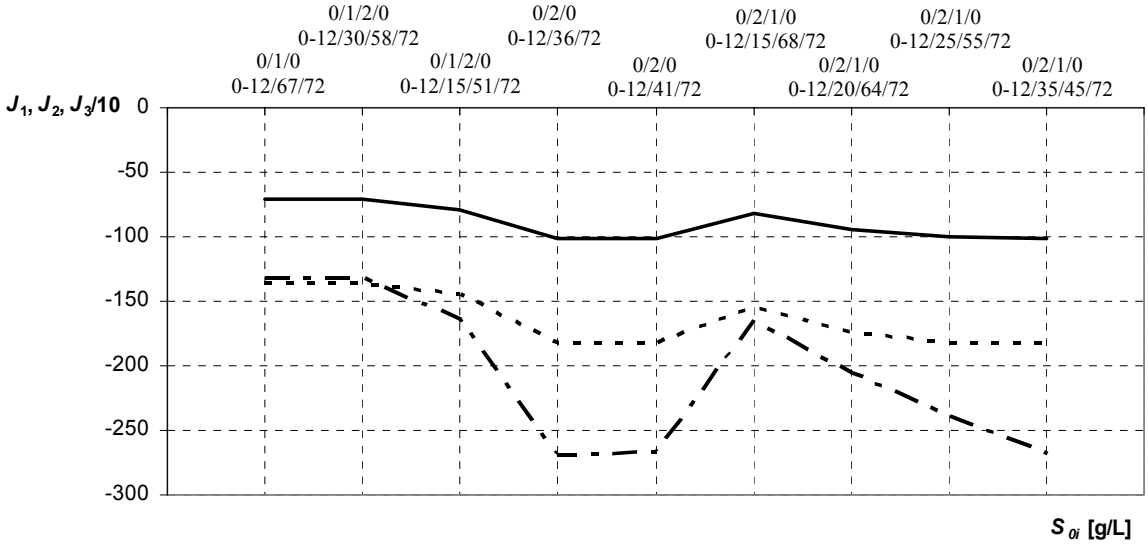

Fig. 3 J_1 ——, J_2 ----, $J_3/10$ -·-·-

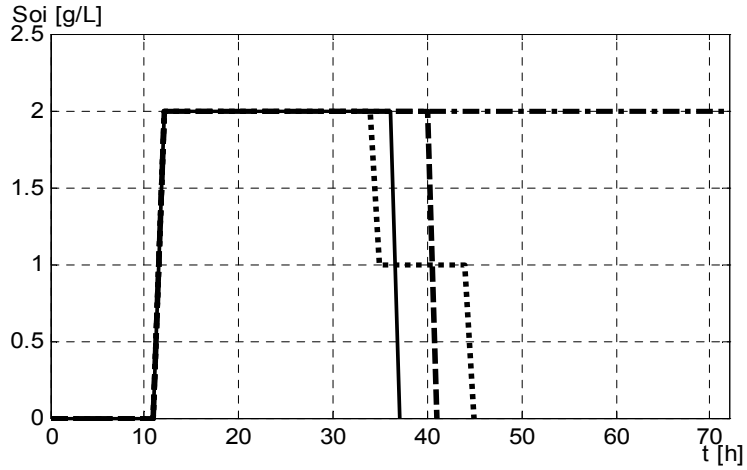

Fig. 4 Feeding profiles
(----, ····· , —— optimal according to J_1, J_2, and J_3, respectively; -·-·- maximum enzyme)

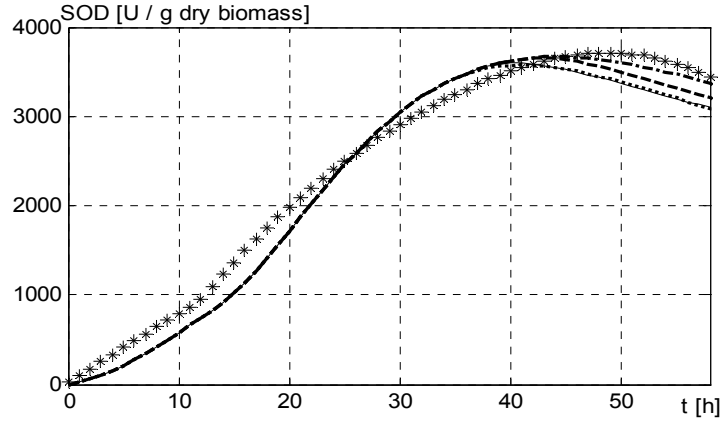

Fig. 5 Maximum of the enzyme according to different profiles
(---- ,····· ,—— optimal according to J_1, J_2, and J_3, respectively; -·-·- maximum enzyme; * real experimental data)

Conclusion

The optimisation of a fed-batch process for the intracellular enzyme superoxide dismutase production was done. It is based on theoretical knowledge for the optimal control of fed-batch biotechnological processes. Experiments with the SOD-producing fungal strain *H. lutea* 103 in a 12 l bioreactor were made. Optimal profiles for glucose feeding based on three optimisation criteria were developed, using nonlinear optimisation methods.

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