Modeling and Fuzzy Optimization of Whey Fermentation by *Kluyveromyces marxianus var. lactis MC 5*

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In this paper lactose oxidation from natural substratum in fermentation of *Kluyveromyces marxianus var. lactis MC 5* on basis of real experimental data is modeled. The batch process is used for identification of the parameters of the model. The model parameters are identified with non-linear regression technique with assistance of a computer program which minimizes the deviation between the model prediction and the actual batch experimental data. The model of fed-batch process is used for optimization. The optimization of the process is made by a fuzzy optimization method. An optimal profile of the impeller speed, the gas flow rate, and the substratum floating rate are obtained.

Key words:
Modeling, lactose oxidation, non-linear regression, fuzzy optimization, whey fermentation

Introduction

The fermentation of lactose oxidation from natural substratum in fermentation of *Kluyveromyces marxianus var. lactis MC 5*8 uses non-conventional whence for receiving of unicellular protein. This process is not well studied. Therefore, it does not exist a general mathematical model of the microbial synthesis, because of the extreme complexity and great variety of living activity of the microorganisms, although, various models of biotechnological process and fermentation of different parts of the whey exist.[10] On this cheese whey, which is a waste product at the production of white brine cheese, oneself with what oneself reception one close cycle.

Because of all that, it is a very important problem its optimization.

A new method for rendering these uncertainties is the fuzzy sets theory.[1–3] The aim of this paper is development of a "*flexible*" model and optimization of a fed-batch process of whey fermentation by a application of the fuzzy set theory.[9,11,12]

Materials and methods

The strain *Kluyveromyces marxianus var. lactis MC5* is cultivated under the following conditions:[1]

1. Nutrient medium with basic component:

Whey ultra filtrate with lactose mass concentration is *44* kg m^{-3}. The ultra filtrate is derived from whey separated in production of white cheese and deproteinisation by ultra filtration on *LAB 38 DDS* with membrane of the type *GR 61 PP* under the following condition:

Temperature	T = 40 – 43 °C;
Input pressure	p_{in} = 0.65 MPa;
Output pressure	p_{out} = 0.60 MPa.

The ultra filtrate is used in native condition with lactose concentration 44 kg m^{-3}.

Nutrient medium consist of $(NH_4)HPO$	6×10^{-3} kg m^{-3};
Yeast's autolisate	5×10^{-3} kg m^{-3};
Yeast's extract	1×10^{-3} kg m^{-3};
pH	5.0 – 5.2.

The changes of the microbiological process (lactose conversion in yeast's cells to protein) are studied during the strain growth:

a) lactose concentration in fermentation medium in oxidation and assimilation of lactose by Kluyweromyces Marxianus var. lactis MC5 is determined by enzyme methods by UV tests (Boehringer Manheim, Germany, 1983);

b) concentration of cell mass and protein contents are determined on the basis of the nitrogenium contents (Kjeltek system 1028);

c) concentration of the dissolved oxygen in the fermentation medium in the process of oxidation and assimilation of lactose is determined by oxygen sensor.

2. The gas flow rate for aeration is $Q_G = 60\ L\ h^{-1}$ up to the 4th hour and $Q_G = 120\ L\ h^{-1}$ up to the end of the process.

3. Continuous mixing
   $n = 800\ min^{-1}$;

4. Temperature of fermentation
   $T = 29$ °C;

5. Duration of the cultivation
   $t = 12\ h$;

6. Volume of growth media
   $V = 1.2\ L$.

For the measurement of the oxygen concentration in the fermentation middle the oxygen sensor that is produced by LKB firm, is used.

Six fermentations where carried out in aerobic batch cultivation of *Kluyveromyces lactis*. The experimental investigations are carried out on the computer controlled laboratory bioreactor *2L-M* with magnetic coupling.[6]

Modeling of the fermentation process

Model of the batch process

The model of the batch processes includes the dependence between the concentrations of the basic variables of the process: cell mass concentration, lactose concentration, oxygen concentration in the gas phase and in the liquid phase. The model is based on the mass balance equations on the perfect mixing in the bioreactor:

$$\frac{d\gamma_X}{dt} = \mu(\gamma_S, \gamma_{C_L})\gamma_X$$

$$\frac{d\gamma_S}{dt} = -Y_1\mu(\gamma_S, \gamma_{C_L})\gamma_X$$

$$\frac{d\gamma_{C_L}}{dt} = \frac{k_1 a}{(1-\varphi_G)}\left(\frac{\gamma_{C_G}}{m_L} - \gamma_{C_L}\right) - Y_2\mu(\gamma_S, \gamma_{C_L})\gamma_X \qquad (1)$$

$$\frac{d\gamma_{C_G}}{dt} = \frac{k_1 a}{\varphi_G}\left(\gamma_{C_L} - \frac{\gamma_{C_G}}{m_L}\right)$$

The initial conditions are given as follows:

$$\gamma_{X(0)} = 0.2\ \text{kg m}^{-3};\ \gamma_{S(0)} = 44\ \text{kg m}^{-3};$$

$$\gamma_{C_L(0)} = 6.65 \times 10^{-3}\ \text{kg m}^{-3};\ \gamma_{C_G(0)} = 0.21\ \text{kg m}^{-3}.$$

The power input in the fermentation medium can be determined by:[6,7]

$$P_G = 0.21(Q_G/n d^3)^{-0.1} P_L^{0.8},$$
$$P_L = 60.9\rho n^3 d^5 Re^{-0.18}(\delta/d)^{-0.23}. \qquad (2)$$

The gas hold-up and the $k_l a$ can be determined by:

$$\varphi_G = P_G/P_L = a_1(Q_G/n d^3)^{b_1}$$
$$k_1 a = a(P_G/V)^b W_G^c \qquad (3)$$

The constants in (3) are determined with experimental investigations.

Evaluation of the model parameters

The evaluation of the parameters in the model (3) is based on a development algorithm and a program for non-linear regression analysis. The validations of the parameters are done on basis of the statistical criteria: the Fisher quotient – $F_{N-1,1}$ and the correlation coefficient – R^2_{N-2}. The theoretical Fisher quotient and the correlation coefficient are taken from F and R^2 tables[8] with 95 % confidence for the whole experimental set.

The coefficients in models (3) after the experimental investigation have values: a = 52.0, b = 0.38, c = 0.23, a_1 = 0.53 and b_1 = –0.014. The computed and the theoretical correlation coefficients for $k_l a$ have values: R_E^2 = 0.998 and R_6^2 = 0.954. The computed and the theoretical Fisher quotient are: F_E = 14.00 and $F_{7,1}$ = 5.59.

The computed and the theoretical correlation coefficients for j_G have values: R_E^2 = 0.95 and R^2_{50} = 0.273. The computed and the theoretical Fisher quotient have values: F_E = 4.76 and $F_{51,1}$ = 4.03.

The analysis of the results shows that the models are adequate and they can be used for computing $k_l a$ and gas hold-up.

After testing of basic dependences for specific grown rate the best results shows (the following comparison between R^2 and F):

$$\mu = \frac{\mu_m \gamma_{S^2}}{(K_S + \gamma_{S^2})} \frac{\gamma_{C_L}}{(K_C + \gamma_{C_L} + \gamma_{C_L}^2/K_1)}.$$

The experimental results for the specific grown rate are calculated by: $\mu = \gamma_{\dot{X}}/\gamma_X$.

The numerical solution of the model (1) is done by Runge-Kuta-Feldberg methods from 4–5 range.[4]

The optimization program for direct search of the minimum of a multivariable function is based on the simplex method of Nelder-Mead.[8] The minimization criteria that is used in the program is:[5]

$$\mathrm{SSWR} = \sum_{i=1}^{N}\sum_{j=1}^{m}\frac{\Delta_{i,j}^2}{W_{i,j}^2} \to \min. \qquad (4)$$

where: $\Delta_{i,j} = (X_{i,j}^{\mathrm{M}} - X_{i,j}^{\mathrm{E}})$; $X_{i,j}^{\mathrm{M}}$, $X_{i,j}^{\mathrm{E}}$ – the model and the experimental data points at each variable, respectively; $W_{i,j} = \max_{j}\lfloor X_{i,j}^{\mathrm{E}}, X_{i,j}^{\mathrm{M}} \rfloor$.

The test hypothesis of a zero mean deviation of the model and the experimental data are used for determinating the validation of the model.[8] The mean residual of each variable Δ_j is calculated with following:[5]

$$\Delta_j = \frac{1}{N}\sum_{i=1}^{N}\Delta_{i,j}, \text{ for } j = 1, m. \qquad (5)$$

The variance of the error of a residual S_j is estimated as follows:

$$S_j = \frac{1}{N-1}\sum_{i=1}^{N}(-\Delta_{i,j})^2\Delta_j, \text{ for } j = 1, m.$$

The values of the statistic λ is defined as:

$$\lambda = \frac{(N-m)}{(N-1)}\frac{N}{m}\sum_{j=1}^{m}\frac{\Delta_j^2}{S_j}. \qquad (6)$$

The statistic λ has $F_{m,\,N-m}$ distribution.

The validation of the parameters in the model (1) is done with the help of Student criterion:[8]

$$\chi_j = T(0.05, N_J - 1)\sqrt{\sigma_j}\big/N_J, \qquad (7)$$

where: $\sigma_j = (I-1)^{-1}\sum_{j=1}^{N_J}\sum_{i=1}^{N_F}(x_{i,j} - \bar{x}_j)^2$,

$\bar{x}_j = N_J^{-1}\sum_{i=1}^{N_J}x_{i,j}$, $N_F = 6$, $N_J = 7$.

The Student criterion is defined from tables,[8] $T = 1.943$.

The values of parameters in the model, the calculated Fisher quotients and the correlation coefficients for each variables of model (1), are shown on Table 1.

The computed with the help of the statistics (6) and the theoretical Fisher quotient are: $F_{\mathrm{E}} = 14.20$ and $F_{3,10} = 8.786$.

The results after simulations are shown in Figure 1.

The obtained results (Fisher quotients, Table 1 and Figure 1) show that the model is adequate and it can be used for the optimization of the fed-batch fermentation process.

Table 1 – *The values of the quantities and statistical information of each variable for the model of batch fermentation. The theoretical correlation coefficient and the Fisher quotient have the following values: $R^2_{10} = 0.576$ and $F_{11,3} = 2.852$.*

Quantity	Min	Value $\pm\,\chi_j$, j = 1,…,7	Max
μm	0.8382	0.9394±0.074	1.0286
K_{S}	1.4526	1.5679±0.079	1.5767
K_{C}	1.08×10^{-3}	$(1.7295 \pm 0.390) \times 10^{-3}$	1.30×10^{-3}
K_1	0.3443	0.5705 ± 0.151	0.8012
Y_1	2.0155	2.1803 ± 0.148	2.4944
Y_2	3.092×10^{-3}	$(3.3882 \pm 0.242) \times 10^{-3}$	3.687×10^{-3}
m_{L}	30.31	33.23 ± 2.264	37.17
Variable	Correlation coefficient, R^2_{E}		Fisher quotient, FE
χ_{X}	0.9828		20.1796
χ_{S}	0.9971		72.7882
$\chi_{\mathrm{C_L}}$	0.9823		10.7154

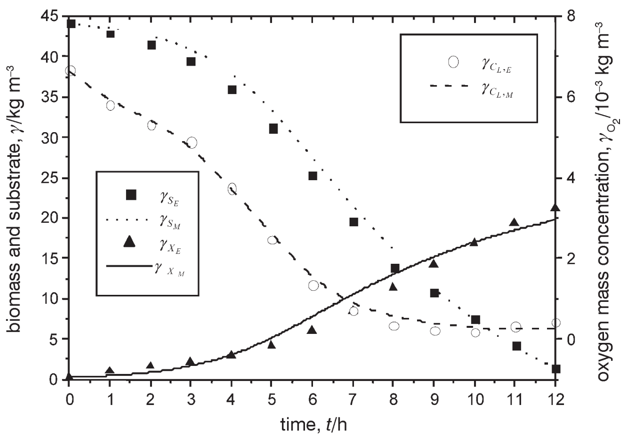

Fig. 1 – *The experimental and the model data of the process*

Model of the fed-batch process

The fed-batch model equations are summarized below:

$$\frac{\mathrm{d}\gamma_{\mathrm{X}}}{\mathrm{d}t} = \mu(\gamma_{\mathrm{S}},\gamma_{\mathrm{C_L}})\gamma_{\mathrm{X}} - D\gamma_{\mathrm{X}}$$

$$\frac{\mathrm{d}\gamma_{\mathrm{S}}}{\mathrm{d}t} = D(\gamma_{\mathrm{S_0}} - \gamma_{\mathrm{S}}) - Y_1\mu(\gamma_{\mathrm{S}},\gamma_{\mathrm{C_L}})\gamma_{\mathrm{X}} \qquad (8)$$

$$\frac{\mathrm{d}\gamma_{\mathrm{C_L}}}{\mathrm{d}t} = \frac{k_1 a}{(1-\varphi_{\mathrm{G}})}\left(\frac{\gamma_{\mathrm{C_G}}}{m_{\mathrm{L}}} - \gamma_{\mathrm{C_L}}\right) - Y_2\mu(\gamma_{\mathrm{S}},\gamma_{\mathrm{C_L}})\gamma_{\mathrm{X}} - D\gamma_{\mathrm{C_L}}$$

$$\frac{\mathrm{d}\gamma_{C_G}}{\mathrm{d}t}=\frac{k_1 a}{\varphi_G}\left(\gamma_{C_L}-\frac{\gamma_{C_G}}{m_L}\right)$$

$$\frac{\mathrm{d}V}{\mathrm{d}t}=Q,\quad D=\frac{Q}{V}$$

where: $\gamma_{S_0}=$ 150 kg m^{-3}; $V(0)$=1.2 L.

The model of the fed-batch fermentation (8) is accepted as a basic in optimization of the process.

Fuzzy optimization of the fed-batch process

The optimization assignment is to find optimal profiles for control variables of the impeller speed – $n(t)$, the gas flow rate – $Q_G(t)$ and the substratum floating rate – $Q(t)$, which maximize the criterion:

$$J(t,\boldsymbol{u})=1-\gamma_{S_0}/\gamma_{S(t)}\rightarrow \max. \qquad (9)$$

The intervals of the control variables are: $n\in[800,1200]$ min^{-1}, $Q_G\in[60,120]$ L h^{-1}, $Q\in[0\div 2.5\times 10^{-3}]$ L h^{-1}; $V(t)\leq 1.3$ L.

The optimization of the process is developed by fuzzy sets. The solution of the formulated optimization problem is accomplished by fuzzy sets theory.[9,11,12] Propose is to use one *flexible* model of the fermentation process. The model of the process (8) is considered as most appropriate but deviations are admissible with small degree of acceptance. It is represented by fuzzy set of the following type "*γ_X, γ_S and γ_{C_L} is come into view approximately by the following relations*", what is given with membership function (Figure 2):[2,3]

$$\beta_i(t,\boldsymbol{u})=1/(1+\eta_i\varepsilon_i^2) \qquad (10)$$

where: $\varepsilon_1=\gamma_{\dot{X}}-\lfloor\mu(\gamma_S,\gamma_{C_L})\gamma_X-D\gamma_X\rfloor$;
$\varepsilon_2=\gamma_{\dot{S}}-\lfloor D(\gamma_{S_0}-\gamma_S)-Y_1\mu(\gamma_S,\gamma_{C_L})\gamma_X\rfloor$;

$$\varepsilon_3=\gamma_{\dot{C}_L}-\left[\frac{k_1 a}{(1-\varphi_G)}\left(\frac{\gamma_{C_G}}{m_L}-\gamma_{C_L}\right)-Y_2\mu(\gamma_S,\gamma_{C_L})\gamma_X-D\gamma_{C_L}\right];\quad i=1,\ldots,3.$$

The admissible diversions from the basic model (8) are given by the parameters η_i, $\eta_i=1$.

The prepositional "*flexible*" model of process reflects the better influence of all good values of the kinetic variables.

Fuzzy criteria from the following type: "*J(t,**u**) to be in possibility higher*", is formulated and presented with the subsequent membership function (Figure 3):[2,3]

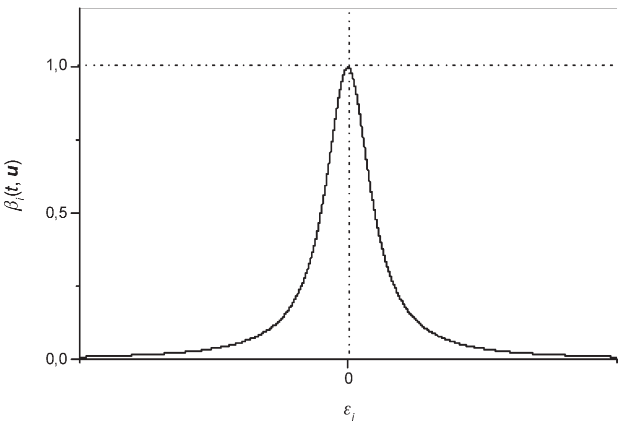

Fig. 2 – *General memberships function of the model*

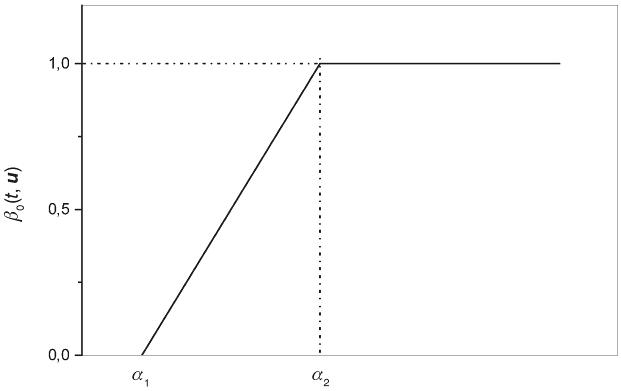

Fig. 3 – *Memberships function of fuzzy criteria*

$$\beta_0(t,\boldsymbol{u})=\begin{cases}0; & J(t,\boldsymbol{u})<\alpha_1\\ \dfrac{J(t,u)-\alpha_1}{\alpha_2-\alpha_1}; & \alpha_1\leq J(t,\boldsymbol{u})\leq\alpha_2\\ 1; & J(t,\boldsymbol{u})>\alpha_2\end{cases} \qquad (11)$$

The following optimization problem from the class of fuzzy mathematical programming problem is formulated:[9,11,12]

$$J(t,\boldsymbol{u})\cong 1-\gamma_{S_0}/\gamma_{S(t)}\rightarrow \widetilde{\max}, \qquad (12)$$

where "$\widetilde{\max}$" means *"in possibility maximum"*; "≅" means "*is come into view approximately in following relation"*.

A non-iterative algorithm for problem solution

The fuzzy set of solution is presented with membership function of the criteria $\hat{a}_0$ and model β_D:[2–3]

$$\beta_D(t,\boldsymbol{u})=(1-\gamma)\prod_0^3\beta_i^{\theta_i}(t,\boldsymbol{u})+\gamma\left\{1-\prod_0^3(1-\beta_i(t,\boldsymbol{u}))^{\theta_i}\right\} \qquad (13)$$

The fuzzy set of solution is determined from the following relations:[2–3]

$$\boldsymbol{u}^0 = \sum_{i=1}^{K} v_i \boldsymbol{u}_i \,, \; v_i = \sum_{i=1}^{I} \frac{\beta^{\theta}_{\mathrm{D}_i}(t,\boldsymbol{u})}{\sum\limits_{j=1}^{J} \beta^{\theta}_{D_j}(t,\boldsymbol{u})} \,, \tag{14}$$

$$i = 1, \; \ldots, \; K; \quad J = K^q.$$

For the obtained model, an effective algorithm for process optimization is synthesized by using fuzzy sets. The following non-iterative algorithm is submitted:

The fuzzy sets of the model $\beta_i(\mathbf{u})$ and the criteria $\beta_0(t,\mathbf{u})$ from (10)–(11) have come into view;

The fuzzy set of solution $\beta_D(t,\mathbf{u})$ from (13) is determined;

The solution $\boldsymbol{u}^0$ from (14) is determined in the end.

With this algorithm optimal profiles of the impeller speed, the gas flow rate, the substratum flow rate and criterion are received, and they are shown on Figure 4.

The results for the biomass and the substrate mass concentration before and after optimization are shown on Figure 5.

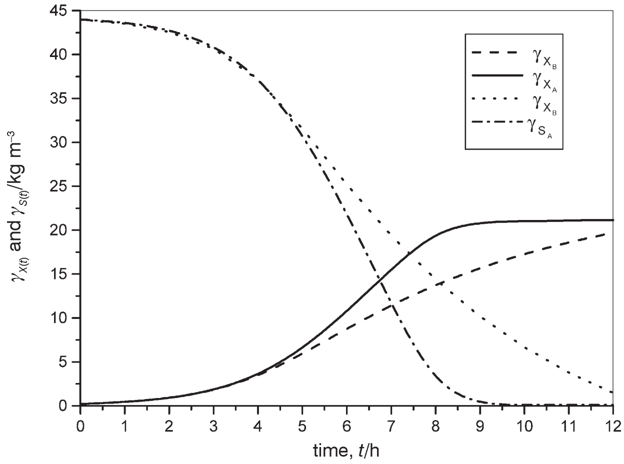

Fig. 5 – *Optimal results of the biomass and the substrate concentration before and after optimization*

Results and discussion

The present results make clear, that the prepositional model of the fermentation process for receiving unicellular protein from natural substratum in fermentation of *Kluyveromyces marxianus var. lactis MC 5*, is adequate (Calculated Fisher quotient, Table 1 and Figure 1).

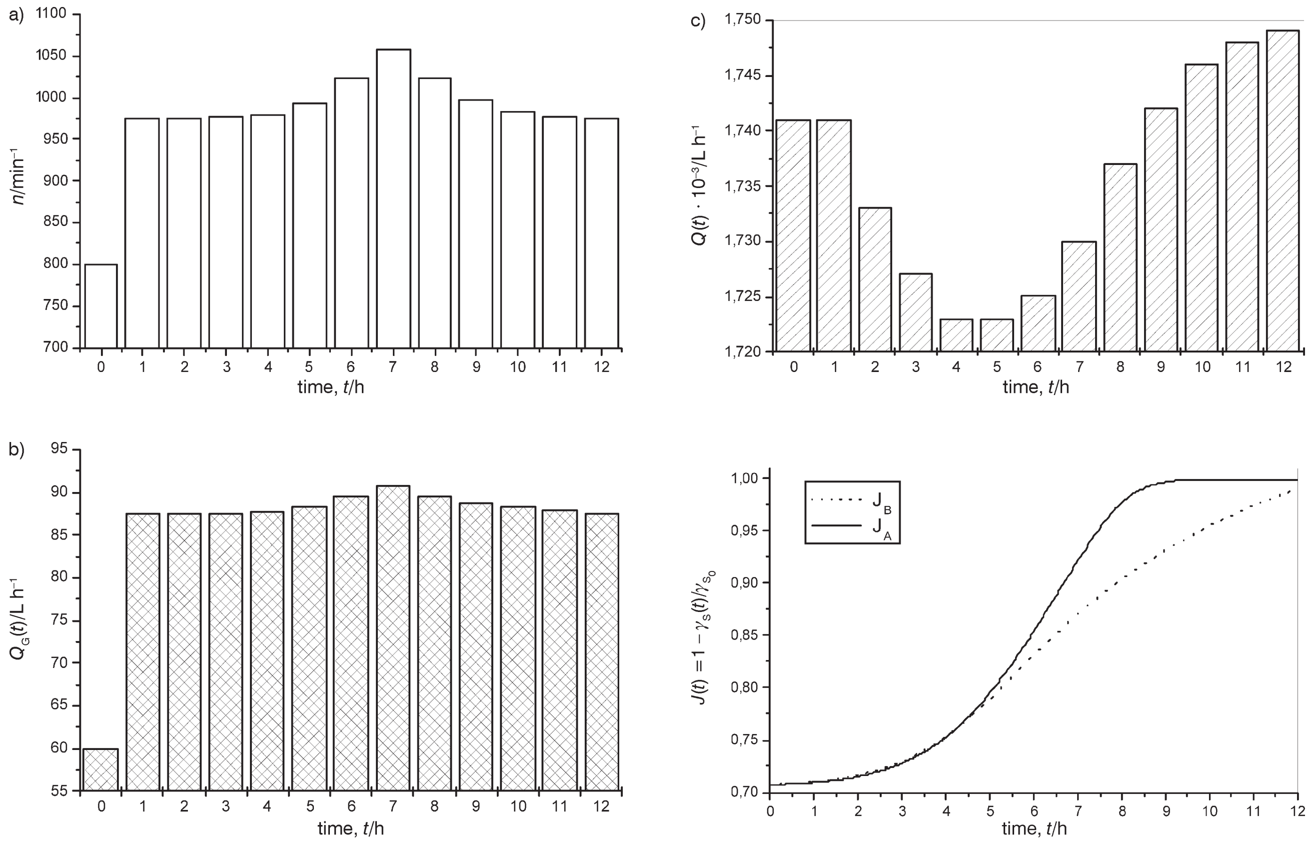

Fig. 4 – *Optimal profiles of the control variables and the criterion:*
a) optimal profile of impeller speed; b) optimal profile of gas flow rate; c) optimal profile of substratum flow rate; d) optimal results of criterion.

From Figure 4 and Figure 5 is shown that after the development optimization of the fed-batch process the leading time of the process can be decreased from 12 to 9 hours. The value of the criterion of the optimization has risen at 9^{-th} hour from 0.932 before the optimization to 0.999 after the optimization, i.e. 7.2 %. The quantity biomass before and after the optimization has risen respectively from 15.709 g L^{-1} to 20.95 g L^{-1}, i.e. 33.4 %.

The quantity of the substrate before and after optimization has decreased respectively from 10.185 kg m^{-3} to 0.125 kg L^{-1}, i.e. more than 80 times, that is a good premise for increasing of the effectiveness use of the substrate.

Conclusions

In this paper on the basis of real experimental data an adequate model of the process by a simulation program is obtained. An adequate model for laboratory scale whey fermentation is developed, and the model parameters for an effective and reliable reactor dynamic, are identified. Model parameters are identified by non-linear regression technique assisted by computer program. The statistical validity of the model indicated confidence on the prediction of the model.

For obtained model using fuzzy optimization an effective algorithm for process optimization is synthesized. The presented "*flexible*" model of fed-batch cultivation reflects in higher way the influence of the kinetic parameters of process on the optimization criteria.

The received optimal profiles of the impeller speed, the gas flow rate, and the substratum floating rate, increase the quantity biomass and the productivity of the process.

This method shows very well results comparison with the classical methods for assignment optimum criterion.

Non-conventional substrate, whence for receiving of unicellular protein, has been presented in this paper. Using of this cheese whey, which is waste product at the production of white brine cheese, oneself with what oneself reception one close cycle.

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Nomenclature

D – dilution rate, h^{-1}
d – impeller diameter, m
D_B – bioreactor diameter, m
$F_{m,N-m}$, F_E – theoretical and experimental Fisher quotients
K – number of discrete values for $\boldsymbol{u}$.
K_C, K_S – Monod's saturation constant for oxygen and substrate, kg m^{-3}
K_I – inhibition constant, kg m^{-3}
$k_l a$ – volumetric gas-liquid mass-transfer coefficient, h^{-1}
m_L – Henry's law constant
n – impeller speed, min^{-1}
N_F – number of the fermentation
N_J – number of the parameters of the model
P_G – power input in fermentation medium with aeration, W
P_L – power input in fermentation medium without aeration, W
Q – feed flow rate, L h^{-1}
q – size of vector u $(q = 3)$
Q_G – gas flow rate, L L^{-1} h^{-1}
Re – Reynolds number
T – Student criterion
t – time, h
$\boldsymbol{u}$ – vector of control variables, $\boldsymbol{u} = \boldsymbol{u}[n, Q_G, Q]$
V – volume, L
$W_{i,j}$ – weight functions
v_G – gas flux, m^3 m^{-2} s^{-1}, $v_G = 4\,Q / \pi D_B^2$
Y_1, Y_2 – yield coefficients, kg kg^{-1}

Greek Letters

α_1, α_2 – the fuzzy sets parameters
γ_S – mass concentration of substrate, kg m^{-3}
γ_{S_0} – initial substrate mass concentration, kg m^{-3}
γ_{C_G} – dissolved oxygen mass concentration in gas-phase, kg m^{-3}
γ_{C_L} – dissolved oxygen mass concentration in liquid phase, kg m^{-3}
γ_X – concentration of the biomass, kg m^{-3}
δ – eccentricity of stirrer toward its rotation axis, m
ρ – liquid density, kg m^{-3}
γ – parameter, characterizing the compensation degree
μ – specific grown rate of biomass, h^{-1}
ε_G – gas hold-up, %
β_i – membership functions, $i = 0,\dots,3$
θ_i – parameters, given of weights of $\hat{a}_i(t,\boldsymbol{u})$, $i = 0,\dots,3$.
ε_i – the parameters of the fuzzy model
μ_{max} – maximal grown rate of biomass, h^{-1}

Subscript

B, A – before and after optimization
E, M – experimental and model data points
m – number of process variables
N – number of data points

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