Fuzzy Optimization of the Biosynthesis of *L-lysine*

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Through using of the fuzzy optimization, an effective algorithm for process optimization is synthesized. The optimization is applied to a fermentation process of the *L-lysine* production in batch conditions. The presented *flexible* model of the batch cultivation reflects in higher way the influence of the kinetic parameters of the process on the optimization criterion. For the first time the initial conditions of a fermentation process of the *L-lysine* production are optimized with the help of the fuzzy sets theory and by investigation of the parameters of the fuzzy sets. After that, the received initial conditions are used for dynamic optimization of the process. The dynamic optimization is realized also by fuzzy sets theory. With these applications the effectiveness is increased – the quality of the *L-lysine* production at the end of the process grows more than 70 %.

Key words:
Biosynthesis, flexible model, optimization, fuzzy sets

Introduction

The *L-lysine* is an irreplaceable amino acid whose contents in the animal protein is high, but in the vegetal protein is relatively low. It is ascertained that the average vegetal contribution of the *L-lysine* (about 30 %) is not adequate for the animal organisms[9]. The insufficient quantity of *L-lysine* in the fodders reduces biological value of the fodder doses, decreases the mass increase and the further productivity of the agricultural animals, raises the quantity fodder used for a kilogram increase and reduces the quality of the animal products.

Expect in the animal husbandry, the *L-lysine* finds application in the food processing industry, in the medicine as a component of the infusion solutions (blood substitutes), also as strengthening patent medicines.

The most effective and cheapest method for the *L-lysine* biosynthesis (in biological active form) is the microbiological method by a direct fermentation.[9]

Usually, at the start of the bioprocess the initial condition of the fermentation are given and they are not investigated subsequently. It proved that for increase of the quality of the process they had materially meaning. Different methods were applied to determine this static optimization.[6] The applying of these method often does not lead to the desired results, because of availability of many and different parameters of the bioprocess.

Development of a model that can reflect the influence of all parameters, under the biosynthesis process of *L-lysine* in the bioreactor, is practicaly impossible and inexpedient. Because of the neglecting of a series of factors which character is not stochastic, thus the defined values of the parameters are distinguished from really optimal. One method for reading of this indefiniteness, as a result of neglecting these factors, is the application of the fuzzy sets theory.[10]

One method for fuzzy optimization[1,2] is also successfully applied for dynamic optimization of a fed-batch process of *E. coli* fermentation and lactose oxidation from natural substratum in a fermentation of *Kluyveromyces marxianus var. lactis MC 5*.[7,8] In contrast to different methods,[3] it allows directly (non-iterative) determination of the problem of static and dynamic optimization.

The aims of this investigation are development of a "*flexible*" model for optimization of the initial conditions of a batch fermentation process for *L-lysine* production with application of the fuzzy sets theory, investigation of the parameters of the fuzzy sets, and further dynamic optimization.

Experimental investigation and model of the process

The experimental investigations are done in a 15 L bioreactor that is included in an Automatic Control System. The Automatic Control System is flexible and includes control of the following quan-

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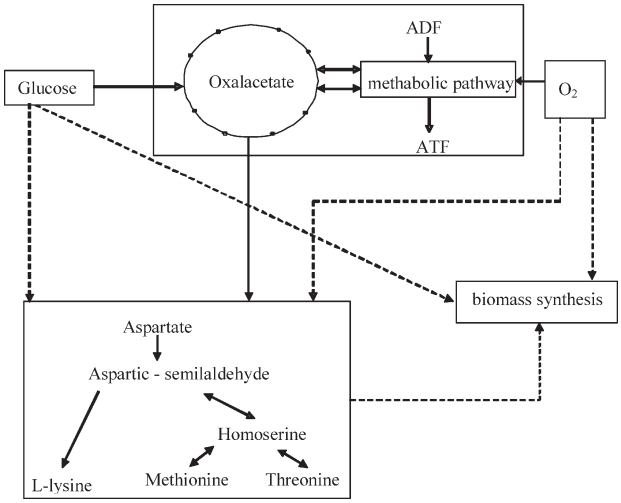
tities of the process: rotation speed, oxygen partial pressure, temperature, *pH*, foam level, gas flow rate, flow rates of the main substance. The process is leaded in the next conditions:

– temperature T = 30 °C;
– pH pH 6.8 – 7.6;
– p_{O_2} p_{O_2} = 20 – 30 %;
– gas flow rate Q_G = 60 L h^{-1};
– rotation speed n = 450 min^{-1};
– maximum bioreactor volume 15 L.

The model of the batch processes includes the dependences between the concentrations of the basic variables of the process: cell mass concentration (bacteria *Brevibacterium flavum*), substrate mass concentration, *L-lysine*, *threonine* mass concentration and oxygen mass concentration in the liquid phase. The general scheme of the *L-lysine* is shown in Figure 1.



F i g . 1 – *A general scheme of the L-lysine biosynthesis*

The mathematical model of the process is based on the mass balance equations, as perfect mixing in the bioreactor is accepted. The model of the process has the following type:[9]

$$\frac{d\gamma_X}{dt} = \mu\gamma_X$$

$$\frac{d\gamma_S}{dt} = -k_5\mu\gamma_X - k_6\Gamma - k_7\eta\gamma_X$$

$$\frac{d\gamma_{Tr}}{dt} = -k_{13}\mu\gamma_X \qquad (1)$$

$$\frac{d\gamma_{C_L}}{dt} = k_1 a(\gamma_C - \gamma_{C_L}) - k_{14}\mu\gamma_X - k_{15}\gamma_X - k_{16}\eta\gamma_X$$

$$\frac{d\gamma_L}{dt} = \eta\gamma_X$$

where:

$$\mu = \frac{k_1\gamma_{Tr}\gamma_{C_L}}{(k_2+\gamma_{Tr})(k_3+\gamma_{S_0}-\gamma_S)(k_4+\gamma_{C_L})};$$

$$\eta = \frac{k_8\gamma_S\gamma_{C_L}}{(k_9+\gamma_S)(k_{10}+\gamma_S)(k_{11}+\gamma_{C_L})(k_{12}+\gamma_{C_L})}.$$

The initial conditions of the process are:[9]

$$\gamma_{X(0)} = 3.0 \text{ g L}^{-1}, \quad \gamma_{S(0)} = \gamma_{S_0} = 100.0 \text{ g L}^{-1},$$

$$\gamma_{Tr(0)} = 80.0 \text{ g L}^{-1}, \quad \gamma_{C_L(0)} = 6.1 \text{ g L}^{-1}.$$

The numerical solution of the model (1) is done by the Runge-Kuta-Feldberg method in the range of 4–5.[5,7]

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]

$$\text{SSWR} = \sum_{i=1}^{N}\sum_{j=1}^{m}\frac{\Delta_{i,j}^2}{W_{i,j}^2} \rightarrow \min,$$

where: $\Delta_{i,j} = (X_{i,j}^M - X_{i,j}^E)$; $X_{i,j}^M, X_{i,j}^E$ – the model and the experimental data points to each variables respectively; $W_{i,j} = \max_j \left\lfloor X_{i,j}^E, X_{i,j}^M \right\rfloor$.

The test of the hypothesis of a zero mean deviation of the model and the experimental data is used for determination of the validation of the model.[7] The mean residual of each variable Δ_j is calculated with following:

$$\Delta_j = \frac{1}{N}\sum_{i=1}^{N}\Delta_{i,j}, \quad \text{for } j = 1, m.$$

The variance of the error of a residual S_j is estimated with following:

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

The value of the statistic λ is defined by:

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

The statistics χ has $F_{m,\,N-m}$ distribution.[7]

The computed values with the help of the statistics χ and the theoretical Fisher quotient are: χ = 19.38 and $F_{5,4}^T$ = 5.19. The present results show that the prepositional model of the biosynthesis of the *L-lysine* is adequate (statistics $\chi > F_{5,4}^T$).

The obtained coefficients of the model have the next values:

$$k_1 = 20.80,\ k_2 = 42.00,\ k_3 = 28.00,\ k_4 = 1.1,$$
$$k_5 = 1.01,\ k_6 = 0.07,\ k_7 = 0.51,\ k_8 = 62.0,$$
$$k_9 = 28.0,\ k_{10} = 37.0,\ k_{11} = 4.0,\ k_{12} = 0.12,$$
$$k_{13} = 6.1,\ k_{14} = 448.0,\ k_{15} = 22.0,\ k_{16} = 209.0;$$
$$k_l a = 120.0.$$

The results after simulations are shown in Figure 2.

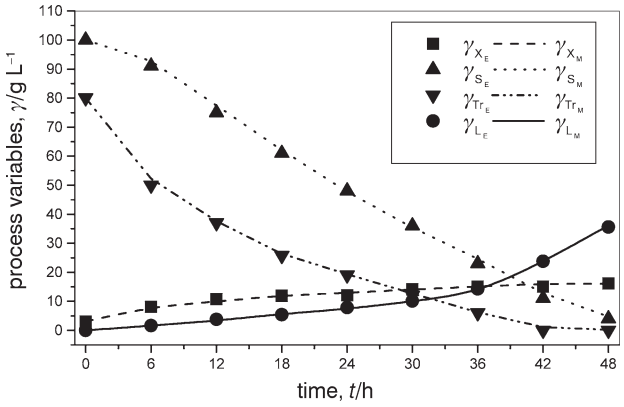


Fig. 2 – *Experimental data and simulation data of the model*

The model of the process (1) is used as a basic for optimization of the initial conditions of the kinetic variables and further dynamic optimization.

Fuzzy optimization of the initial conditions of the process

The optimization problem is formulated in the next way: to be found such initial conditions of the kinetic variables $\gamma_{X(0)}$, $\gamma_{S(0)}$, $\gamma_{Tr(0)}$ and $\gamma_{C_L(0)}$ maximizing the quantity of the useful product *(L-lysine)* so that at the end of the process to be increased:

$$J(\boldsymbol{u}) = \int_0^T \gamma_{L(t)} \mathrm{d}t \rightarrow \max \qquad (2)$$

The vector of the control variables are of the type: $\boldsymbol{u} = \boldsymbol{u}[u_1, u_2, u_3, u_4]$, where:

$u_1 \equiv \gamma_{X(0)}$, $u_2 \equiv \gamma_{S(0)}$, $u_3 \equiv \gamma_{Tr(0)}$ and $u_4 \equiv \gamma_{C_L(0)}$.

Therefore, in this paper the use of a "*flexible*" model is offered[1,2] which reflects more fully all possible values of the criterion and the control variables. The initial conditions and the model of the process (1) are considered as most appropriate, but deviations are admissible with small degree of acceptance. This is represented by fuzzy set with the following membership function[1] (Figure 3a):

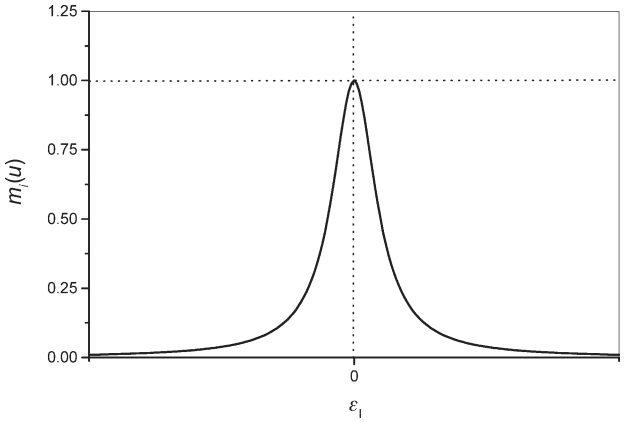
$$m_i(\boldsymbol{u}) = \frac{1}{1 + \varepsilon_i^2(\boldsymbol{u})}, \qquad (3)$$

where:

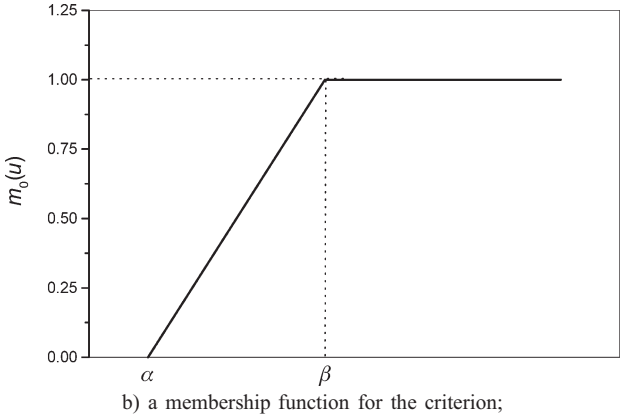
$$\varepsilon_1 = \dot{\gamma}_X - \mu\gamma_X$$
$$\varepsilon_2 = \dot{\gamma}_S + k_5\mu\gamma_X + k_6\Gamma + k_7\eta\gamma_X$$
$$\varepsilon_3 = \dot{\gamma}_{Tr} + k_{13}\mu\gamma_X$$
$$\varepsilon_4 = \dot{\gamma}_{C_L} - k_1 a(\gamma_{\dot{C}} - \gamma_{C_L}) + k_{14}\mu\gamma_X + k_{15}\gamma_X + k_{16}\eta\gamma_X$$
$$\varepsilon_5 = \dot{\gamma}_L - \eta\gamma_X$$

Fuzzy criterion from the following type: "*The optimization criterion J(u) to be in possibility higher*", is formulated and presented with the subsequent membership function[1,] (Figure 3b):

$$m_0(\boldsymbol{u}) = \begin{cases} 0; & J(\boldsymbol{u}) < a \\ [J(\boldsymbol{u}) - \alpha]/(\beta - \alpha); & a \le J(\boldsymbol{u}) \le \beta \\ 1; & J(\boldsymbol{u}) > \beta \end{cases} \qquad (4)$$



a) membership function for the model



b) a membership function for the criterion;

Fig. 3 – *A membership function for the model and criterion*

The following fuzzy optimization problem from the class of fuzzy mathematical programming problems is formulated.[1–3,7,8]

$$J(u) \cong \int_0^T \gamma_{\mathrm{L}(t)} \mathrm{d}t \rightarrow \tilde{\max} \qquad (5)$$

where:

$\tilde{\max}$ means *"in possibility maximum"*;

$\cong$ – means "*in come into view approximately in following relation"*.

The fuzzy sets of the initial condition are determined as arranged couples compound from the sets of their possible values and their respective membership functions:

$$u_1 = \{\gamma_{\mathrm{X}(0)}, m_1(\boldsymbol{u})\},\ \gamma_{\mathrm{X}(0)} = \{3.0, 3.5, 3.6, 3.7, 4.0\}$$

$$u_2 = \{\gamma_{\mathrm{S}(0)}, m_2(\boldsymbol{u})\}, \gamma_{\mathrm{S}(0)} = \{100.0, 110.0, 120.0, 125.0, 130.0\}$$

$$u_1 = \{\gamma_{\mathrm{X}(0)}, m_1(\boldsymbol{u})\},\ \gamma_{\mathrm{X}(0)} = \{3.0, 3.5, 3.6, 3.7, 4.0\}$$

The fuzzy set of the decision is presented by a membership function m_D that is conjunction from the membership functions of criterion $m_0(\boldsymbol{u})$ and the model $m_i(\boldsymbol{u})$:

$$m_{\mathrm{D}}(\boldsymbol{u}) = m_0(\boldsymbol{u}) \text{ AND } m_1(\boldsymbol{u}) \qquad (6)$$

The conjunction of the fuzzy set is usually presented by the so called "*min- agregator*":

$$m_0(\boldsymbol{u}) \text{ AND } m_i(\boldsymbol{u}) = \min\,[m_0(\boldsymbol{u}),\ m_i(\boldsymbol{u})] \qquad (7)$$

This performance is rather strictly. Zimmermann (this is a classical well-known method) introduces the so called "*Compensatory AND"* real performance for more reality of the aggregation between the criterion and the limits.

The "*Compensatory AND"* is used for the determination of the m_{D}:

$$m_{\mathrm{D}}(\boldsymbol{u}) = (1-\lambda)\prod_{i=0}^{5} m_i^{\theta_i}(\boldsymbol{u}) + \lambda\left\{1 - \prod_{i=0}^{5}(1 - m_i(\boldsymbol{u}))^{\theta_i}\right\}. \qquad (8)$$

Filev and *Yager*[4] have extended this idea by considering *defuzzification* BADD as a selection of an expected value given the probability distribution derived from the membership function. The use of expected values instead of random values avoid the noise effects, which are usually undesirable when working with sequences, e.g. in the control of time continuous processes. The method is applied to the fuzzy optimization problem and besides all possible decisions with different weights, given by a parameter θ_i are read:

$$\boldsymbol{u}^0 = \sum_{i=1}^{d} v_i \boldsymbol{u}_i; \qquad v_i = \frac{m_{\mathrm{D}_i}^{\theta_i}(\boldsymbol{u})}{\sum_{j=1}^{n} m_{\mathrm{D}_j}^{\theta_j}(\boldsymbol{u})}; \qquad i = 1, \ldots, d; \qquad n = d^m. \qquad (9)$$

This method allows direct (non-iterative) determining of the optimization problem. The non-iterative algorithm has following type:

1. Induction of the initial values of the parameters in the models;

2. Induction of the fuzzy set of the model $m_i(\boldsymbol{u})$ and the criterion $m_0(\boldsymbol{u})$;

3. Determination of the optimisation criterion $J(\boldsymbol{u})$;

4. Determination of the fuzzy set $m_D(\boldsymbol{u})$;

5. Determination of the fuzzy set of the decision $\boldsymbol{u}^0$;

6. Print results;

7. End.

A program on FORTRAN 77 is developed that realizes the offered algorithm.

The found optimal initial conditions using the described algorithm for fuzzy optimization, have the next values:

$$\gamma_{\mathrm{X}(0)} = 3.43 \text{ g L}^{-1}, \quad \gamma_{\mathrm{S}(0)} = \gamma_{\mathrm{S}_0} = 120.0 \text{ g L}^{-1},$$

$$\gamma_{\mathrm{Tr}(0)} = 83.81 \text{ g L}^{-1}, \quad \gamma_{\mathrm{C_L}(0)} = 6.91 \text{ mg L}^{-1}.$$

For receiving of better results at the optimization of the initial conditions, the influence of the parameters of the fuzzy sets on the solution of the optimization problem will be investigated. In this way, their values will be chosen by the criterion at which they have maximal value.

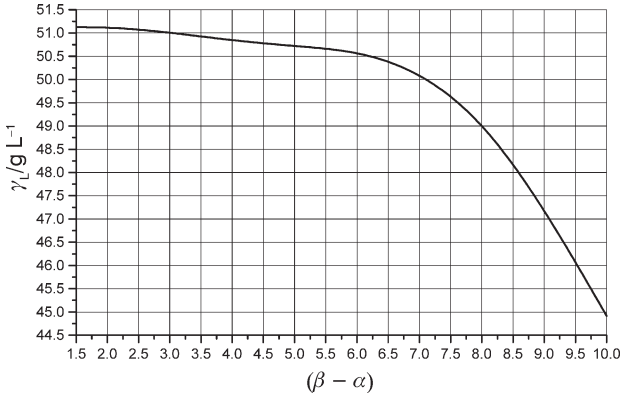
The next results illustrate the dependence of the received solution on the following parameters: *(β-α), λ, θ and* $\theta_{\mathrm{criterion}}/\theta_{\mathrm{model}}$ (Figure 4).

From the shown figures the following conclusions can be done:

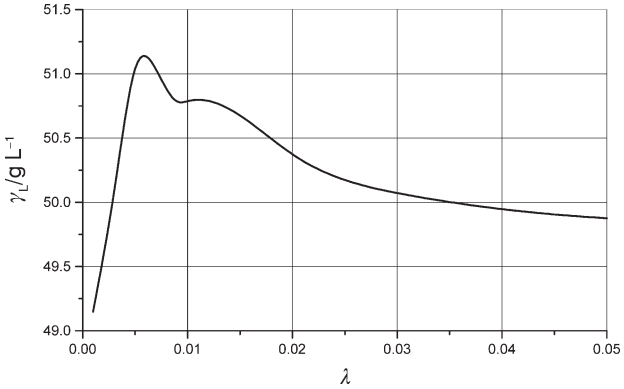
1. The result is better as more strictly the fuzzy criterion is given (a steeper membership function, i.e. less value of β–α), (Figure 3a, *for* $\lambda = 0.005$, $\theta = \theta_i = 1$);

2. The result is higher when the operator for flexible conjunction inclines to the so called "product – operator" (at small λ), i.e. when the degree of the logical compensation is low (Figure 4b, *for* β–$\alpha = 5$, $\theta = \theta_i = 1$);

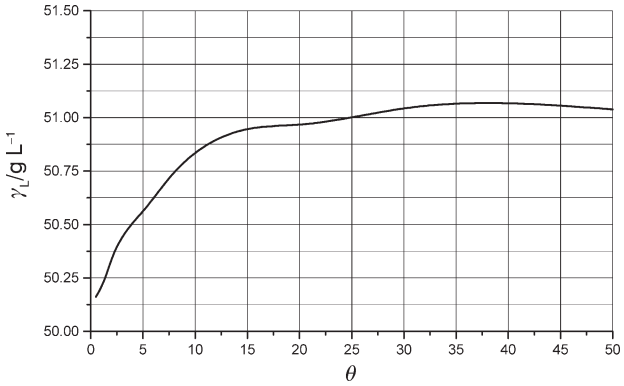
3. The result is increase at growth of the θ, i.e. when the operator inclines to *defuzzification* on maximal value (Figure 4c, *for* β–$\alpha = 5$, $\lambda = 0.005$ and $\theta = \theta_i = 1$);



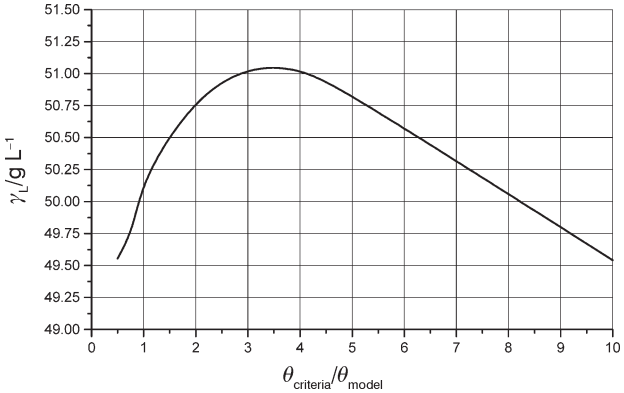
a) Dependence of the solution from (β-α)



b) Dependence of the solution from λ



c) Dependence of the solution from θ;



d) Dependence of the solution from $\theta_{criterion}/\theta_{model}$

F i g . 4 – *Influence of the fuzzy sets on the solution*

4. The dependence of the result from the ratio is more complex $\theta_{criterion}/\theta_{model}$, but it is noticed that it is higher when this ratio is equally to 3.5, i.e. when the fuzzy criterion has 3.5 times bigger mass from the model (Figure 4d, *for* β–α = 5, λ = 0.005 and θ = 1).

As it is noticed from developed investigation for assessment of the influence of the parameters of the fuzzy sets on the solution, the optimal initial conditions are different and their values are:

$$\gamma_{X(0)} = 3.76 \text{ g L}^{-1}, \quad \gamma_{S(0)} = \gamma_{S_0} = 120.0 \text{ g L}^{-1},$$

$$\gamma_{Tr(0)} = 83.46 \text{ g L}^{-1}, \quad \gamma_{C_L(0)} = 7.0 \text{ mg L}^{-1}.$$

These initial will be used for fuzzy dynamic optimization of the *L-lysine* biosynthesis. As a control variable the k_la will be used.

Fuzzy dynamics optimization of the process

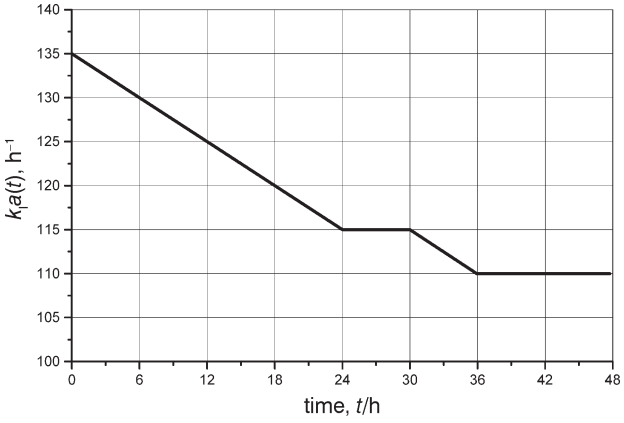
The intervals of the control variable are $90.0 \leq k_La \leq 150.0$. The criterion of the dynamic optimization problem has type:

$$J(t,k_1a) \cong \int_0^T \gamma_{L(t)}(t,k_1a)\mathrm{d}t \rightarrow \tilde{\max} \qquad (10)$$

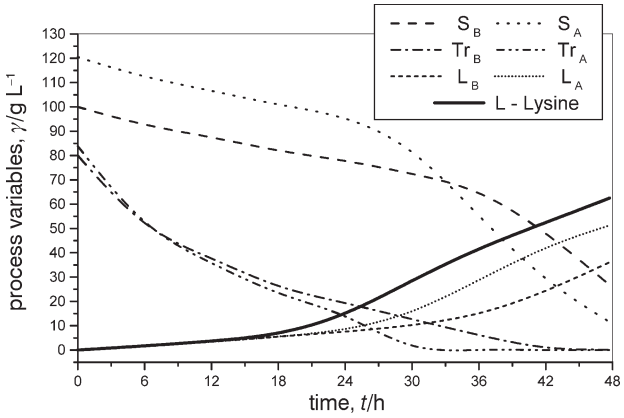
The dynamic optimization problem is solved by the approach presented in the previous point. With this algorithm, optimal profile of the k_la is received and it is shown on Figure 5.

The optimal profiles of the kinetic variables before and after optimization of the initial conditions and optimal profile of the L-lysine before and after dynamic optimization are shown on Figure 6.

From Figure 5 it is noticed augmentation of the *L-lysine* quantity with more than 70 % at the end of the process in comparison without optimization.



F i g . 5 – *Optimal profile of the k_la*



F i g . 6 – *Results before and after static and dynamic optimization, where L-lysine reflects the results after the dynamic optimization*

Conclusion

For the first time the fuzzy sets theory is applied to static optimization of the initial conditions and further dynamic optimization of a batch fermentation process for *L-lysine* biosynthesis. A non-iterative algorithm for fuzzy optimization is synthesized and on its basis a program is developed, that realizes it. For realization of optimization problem the influence of the parameters of the fuzzy sets are investigated. In this way the values of the initial kinetic variable are found.

The received optimal initial conditions of the kinetic variables vastly increase the effectiveness of the process and they are used for dynamic optimization of the fermentation. The dynamic optimization of the process is made with optimal control variable of the volumetric oxygen mass transfer coefficient by fuzzy sets theory. With using this profile, the quality of the process is raised at the end of the process.

The used method allows direct (non-iterative) determining of the problem of the static and the dynamic optimization. The offered approach shows its priority in comparison with different method for optimization and can be successfully applied in another study cases.

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Nomenclature

d – number of discrete values for $\boldsymbol{u}$.
k_i – regression coefficient in the model, i = 1, 16
$k_l a$ – volumetric mass-transfer coefficient, h^{-1}
m – size of vector $\boldsymbol{u}$
m_A – membership functions of the fuzzy set
m_0 – membership functions for the criterion
m_i – membership functions for the model, i = 1,…,5
q – size of vector $\boldsymbol{u}$ (q = 4 and q = 1)
t – time, h
$\boldsymbol{u}$ – vector of control variables, ### and $\mathbf{u}=\mathbf{u}[k_l a]$

Greek letters

α, β – fuzzy sets parameters
ε_i – deviations of the basic models, i = 1,…,5
λ – parameter that characterizes the level of compensation
θ_i – parameters, that give the weights of the $m_i(\boldsymbol{u})$; i = 0,…,5
η – specific consummation rate of substrate, h^{-1}
μ – specific grown rate of biomass, h^{-1}
γ_S – mass concentration of substrate, g L^{-1}
$\gamma_{S(0)}$ – initial mass concentration of substrate, g L^{-1}
γ_X – mass concentration of the biomass, g L^{-1}
$\gamma_{X(0)}$ – initial mass concentration of the biomass, g L^{-1}
γ_{C_L} – dissolved oxygen mass concentration in liquid phase, mg L^{-1}
$\gamma_{C_L(0)}$ – initial dissolved oxygen mass concentration in liquid phase, mg L^{-1}
γ_L – *L-lysine* mass concentration, g L^{-1}
γ_{C^*} – mean oxygen mass concentration, mg L^{-1}
γ_{Tr} – *threonine* mass concentration, g L^{-1}
$\gamma_{Tr(0)}$ – initial *threonine* mass concentration, g L^{-1}
$\dot{\gamma}$ – rate of mass concentration g l^{-1} h^{-1}
Γ – mass concentration rate, g L^{-1} h^{-1}

Subscript

B – before optimization
A – after optimization
E – experimental data
M – model data
l – liquid
L – *L-lysine*

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