A Multiple-objective Optimization of Whey Fermentation in Stirred Tank Bioreactors

Mitko Petrov

Centre of Biomedical Engineering "Prof. Ivan Daskalov" - Bulgarian Academy of Science
105 Acad. G. Bonchev Str., Sofia 1113, Bulgaria
E-mail: mpetrov@clbme.bas.bg

Abstract: A multiple-objective optimization is applied to find an optimal policy of a fed-batch fermentation process for lactose oxidation from a natural substratum of the strain Kluyveromyces marxianus var. lactis MC5. The optimal policy is consisted of feed flow rate, agitation speed, and gas flow rate. The multiple-objective problem includes: the total price of the biomass production, the second objective functions are the separation cost in downstream processing and the third objective function corresponds to the oxygen mass-transfer in the bioreactor. The multiple-objective optimization are transforming to standard problem for optimization with single-objective function. Local criteria are defined utility function with different weight for single-type vector task. A fuzzy sets method is applied to be solved the maximizing decision problem. A simple combined algorithm guideline to find a satisfactory solution to the general multiple-objective optimization problem. The obtained optimal control results have shown an increase of the process productiveness and a decrease of the residual substrate concentration.

Keywords: Multiple-objective optimization, Fuzzy sets, Fuzzy optimal control, Non-iterative algorithm.

Introduction

Multiple-objective optimization is a natural extension of the traditional optimization of a single-objective function. If the multiple-objective functions are commensurate, minimizing single objective function it is possible to minimize all criteria and the problem can be solved using traditional optimization techniques. On the other hand, if the objective functions are incommensurate, or competing, then the minimization of one objective function requires a compromise in another objective function. The competition between multiple-objective functions is a key distinction between multiple-objective optimization and traditional single-objective optimization [9].

Multiple-objective optimization provides a framework for understanding the relationships between the various objective functions and allows an engineer to make decisions on how to trade-off among the objectives to achieve "the best" process performance. It is an inherently interactive algorithm, with the engineer constantly making decisions [4, 6, 14].

Zhou at all [16] have used of a Pareto optimisation technique to locate the optimal conditions for an integrated bioprocessing sequence and the benefits of first reducing the feasible space by the development of a series of windows of operation to provide a smaller search area for the optimisation.

Vera at all [12] have illustrated a general multiple-objective optimization framework of biochemical systems and have applied it optimizing of several metabolic responses involved

in the ethanol production process by using *Saccharomyces cerevisiae* strain. The general multiple-objective indirect optimization method (GMIOM) is based on the use of the power law formalism to obtain a linear system in logarithmic coordinates. The problem is addressed with three variants within the GMIOM: the weighted sum approach, the goal programming and the multi-objective optimization. We have compared the advantages and drawbacks of each one of GMIOM modes. The obtained results have shown that the optimization of biochemical systems was possible even if the underlying process model was not formulated in S-system form and that the systematic nature of the method has facilitated the understanding of the metabolic design and could be of significant help in devising strategies for improvement of biotechnological processes.

Messac at al. [7] have examined optimization problems that can be partitioned into two categories hereby so called "blind" optimization and "physical" optimization. In "blind" optimization the analyst doesn't have knowledge about the physical meaning of the problem, or about the nature of its anticipated solution. In "physical" optimization the decision maker does have substantive knowledge and often clear objectives regarding of the problem aspects that can be modelled in physically meaningful terms. Approximately, all operational research or engineering design problems belong to the second category. This paper explores a new optimization philosophy, linear physical programming, for operational research applications by addressing the distinct issues related to multiple objective optimizations.

Tonnon at all [11] have used interactive procedure to solve multiple-objective optimization problems. A fuzzy set has been used to model the engineer's judgment on each objective function. The properties of the obtained compromise solution were investigated along with the links between the present method and those based fuzzy logic. An uncertainty which has been affecting the parameters is modelled by means of fuzzy relations or fuzzy numbers, whose probabilistic meaning is clarified by random set and possibility theory. Constraint probability bounds that satisfy a solution can be calculated and procedures that consider the lower bound as a constraint or as an objective criterion are presented. Some theorems make the computational effort particularly limited on a vast class of practical problems. The relations with a recent formulation in the context of convex modelling are also pressured.

In the paper of Wang at all [13] a fuzzy-decision-making procedure is applied to find the optimal feed policy of a fed-batch fermentation process for fuel ethanol production using a genetically engineered *Saccharomyces* yeast *1400 (pLNH33)*. The policy consider control variables such as - feed flow rate, feed concentration, and fermentation time. By using an assigned membership function for each of the objectives, the general multiple-objective optimization problem can be converted into a maximizing decision problem. In order to obtain a global solution, a hybrid search method of differential evolution is introduced.

In this study multiple-objective optimization of an aerobic fed-batch cultivation of *Kluyveromyces lactis* MC5 was developed. The single-objective functions reflect of the biomass process productiveness, substrate utilization and oxygen mass-transfer indexes of the bioreactor. The multiple-objective optimization problem was transformed to a problem with a single-objective function by utility functions with weight coefficients for each single criterion.

The objective of this study was to apply a multiple-objective optimization and fuzzy optimal control strategy to determine the optimal policy of a whey fed-batch fermentation process in stirred tank bioreactors with ideal mixing.

Material and methods

Process specific

Six fermentations where carried out in an aerobic fed-batch cultivation of *Kluyveromyces lactis*. A laboratory bioreactor ABR 02M with capacity 2 liters has been used. The strain *Kluyveromyces marxianus var. lactis* MC5 was cultivated under the following conditions (published in details elsewhere [2]):

1. Nutrient medium with basic component – whey ultra filtrate with lactose concentration 44 g/l. The ultra filtrate was collected from whey separated in production of white cheese and deproteinisation by ultra filtration on LAB 38 DDS with GR 61 PP membrane type under the following condition:

   - Temperature T = 40-43 °C;
   - Input pressure P_i = 0.65 MPa;
   - Output pressure P_o = 0.60 MPa.

   The ultra filtrate was used in native condition with lactose concentration 44 g/l. Nutrient medium ingredients were as follows:

   - $(NH)_4HPO$ 0.6 %;
   - yeast's autolisate   5 %;
   - yeast's extract   1 %;
   - pH   5.0-5.2.

2. The gas flow rate was 60 l·h^{-1} up to the 4th hour and 120 1·h^{-1} up to the end of the process under continuous mixing, where n = 800 rpm.

3. Temperature is 29°C.

4. The microbiological process changes (lactose conversion by yeast's cells to protein) were studied during the strain growth:

   - Lactose concentration in fermentation medium was determined by enzyme methods by UV tests (Boehringer Manheim, Germany, 1983);
   - Cell mass concentration and protein content were determined by using Kjeltek system 1028 [10];
   - Dissolved oxygen concentration in the fermentation medium was determined by LKB oxygen sensor.

5. Duration of the cultivation was t_f = 12 hours.

Fed-batch model

The kinetics model of whey fed-batch process have included the measurable variables of the process such as: biomass concentration, substrate concentration and oxygen concentration in the liquid phase [8]:

$$\dot{X} = \mu(S,C)\,X - F\,X/V \qquad (1)$$
$$\dot{S} = F\,(S_{in} - S)/V - Y_1\,\mu(S,C)\,X \qquad (2)$$

$$\dot{C} = \frac{k_l a}{(1 - \varepsilon_G)} \, (C^* - C) - Y_2 \, \mu(S,C) \, X - F \, X / V \tag{3}$$

$$\dot{V} = F / V \tag{4}$$

where: X – biomass concentration, [g·l^{-1}]; S – substrate concentration, [g·l^{-1}]; C – dissolved oxygen concentration, [g·l^{-1}]; C^* – equilibrium dissolved oxygen concentration, [g·l^{-1}]; S_{in} – input feed substrate concentration, [g·l^{-1}]; V – working liquid volume, [l]; F – feed rate, [l·h^{-1}]; Y_1 and Y_2 – yield coefficients [g/g]; $k_l a$ – volumetric liquid mass transfer coefficient, [h^{-1}]; $\mu(S, C)$ – specific growth rate, [h^{-1}];

$$\mu(S,C) = \mu_m \frac{S^2}{(k_S + S^2)} \frac{C}{(k_C + C + C^2 / k_i)},$$

where: μ_m – maximum specific growth rate, [h^{-1}]; k_S, k_C – saturation constants [g·l^{-1}]; k_i – inhibition constant, [g·l^{-1}];

$$k_l a = 52 \left(10^{-3} P / V \right)^{0.38} W_G^{0.23};$$

where: P – power input, $P = 60.9 \rho \, n^3 d^5 \mathrm{Re}^{-0.4}$, [W]; ρ – liquid density, [kg/m^3]; n – agitation speed, [rpm]; d – impeller diameter, [m]; Re – Reynolds number; W_G – gas velocity, $W_G = 4 \, Q / \pi \, D^2$ [m/s]; Q – gas flow rate, [m^3/s]; D – bioreactor diameter, [m]; ε_G – gas hold-up, $\varepsilon_G = 0.53 \left(Q / nd^3 \right)^{-0.014}$.

The initial conditions and the kinetics model coefficients values used in the study were as follows [8]:

$X(0) = X_0 = 0.28$; $S(0) = S_0 = 49.7$, $S_{in} = 67$; $C(0) = C_0 = 6.3 \times 10^{-3}$; $C^* = C_0$; $F(0) = F_0 = 0.01$; $V(0) = V_0 = 1$; $\mu_m = 0.89$; $k_S = 1.62$; $k_C = 3.37 \times 10^{-3}$; $k_i = 0.47$; $Y_1 = 2.25$; $Y_2 = 3.4 \times 10^{-3}$.

System constraints
For the most bioengineering processes have to be applied physical constraints. The bioreactor volume constraint can be described as follows

$$g_1 = V(t) - V_f \le 0 \tag{5}$$

The substrate and oxygen concentrations have to be positive over process time. We have therefore

$$g_2 = -S(t) \le 0 \tag{6}$$

$$g_3 = -C(t) \le 0 \tag{7}$$

In addition, the stoichiometry of the biomass formation from substrate and oxygen must be obeyed, posing two constraints as follows

$$g_4 = \frac{X(t) \, V(t) - X_0 \, V_0}{[V(t) - V_0] S_{in} + S_0 \, V_0 - S(t) \, V(t)} - \frac{1}{Y_1} \le 0 \tag{8}$$

$$g_5 = \frac{X(t)\,V(t) - X_0\,V_0}{\dfrac{k_l a}{(1 - \varepsilon_G)}\,V(t)\,(C^* - C) + C_0\,V_0 - C(t)\,V(t)} - \frac{1}{Y_2} \leq 0 \tag{9}$$

If the constraints in equations (8) and (9) are not included in the optimization problem, an unrealistic predicted value may be found [3].

The control variables constraints (flow rate $F(t)$, agitation speed $n(t)$ and air flow rate $Q(t)$) were as follows:

$$0\ \text{l·h}^{-1} = F_{\min} \leq F(t) \leq F_{\max} = 0.05\ \text{l·h}^{-1}$$
$$600\ \text{rpm} = n_{\min} \leq n(t) \leq n_{\max} = 1000\ \text{rpm}$$
$$0\ \text{l·h}^{-1} = Q_{\min} \leq Q(t) \leq Q_{\max} = 120\ \text{l·h}^{-1}$$

Because the feed rate $F(t)$, agitation speed $n(t)$, and gas flow rate $Q(t)$ are time dependent variables, the optimal control problem can be considered such as an infinite dimensional problem. To solve this problem efficiently, the feed flow rate, agitation speed, and gas flow rate were represented by a finite set of control parameters in the time interval $t_{j-1} < t < t_j$ as follows:

$F(t) = F(j)$, $n(t) = n(j)$, and $Q(t) = Q(j)$ for $j = 1, \ldots, K$ – number of time partitions.

Formulation of multiple-objective optimization problem

The objective of this work was to find optimal feed flow rate, rotation speed, and gas flow rate of the fed-batch process such as the biomass production should be greater than or equal to some threshold value.

According to this statement, the optimization task has been formulated as a multiple-objective decision-making problem. Two requirements have to be satisfied in such a decision-making problem. The first requirement was to find the optimal values of feed flow rate, substrate feed concentration, and fermentation time, and the corresponding optimal objective function value. Such an optimal solution can be obtained by using multiple-objective optimization techniques. On the other hand, the second requirement was to check whether or not the optimal solution should have satisfied the pre-assigned threshold values. If the optimal solution does not satisfy the threshold values, the decision-making has to trade-off some threshold values. The search efforts should be repeated to find another local optimal solution.

This problem is simply called the multiple-objective optimization problem and is expressed as

$$\max_{\mathbf{u}} f_1 = X(t_f)\,V(t_f) - X_0 V_0 \tag{10}$$

$$\max_{\mathbf{u}} f_2 = \frac{S_0 - S(t_f)}{S_0} \tag{11}$$

$$\max_{\mathbf{u}} f_3 = \frac{k_l a\,V}{(1 - \varepsilon_G)\,Q_G}\left(1 - C(t_f)/C^*\right) \tag{12}$$

The first objective function corresponds to the total price of biomass production. The second objective function is the separation cost in downstream processing. The third objective function stands for the oxygen mass-transfer processes in the bioreactor.

The multiple-objective optimization problem was formulated in the following way: to be founded such values of the control variables, united in the vector **u**, for that the vector criterion $Q(\mathbf{u})$, with elements of the separate single-objective function $f_i(\mathbf{u})$, $(i = 1, 2, 3)$ accepts an optimal value and the formulated constrains are satisfied Eqs. (5) - (9):

$$Q(\mathbf{u}) = \left[f_1(\mathbf{u}), \, f_2(\mathbf{u}), \, f_3(\mathbf{u})\right] \quad \rightarrow \quad \max_{\mathbf{u}} \tag{13}$$

$$g_j(\mathbf{u}) \le 0, \quad j = 1, 2, ..., 5$$

For determination of the optimization problem Eq. (17) a utility function method was used for one vector problem:

$$\max_{\mathbf{u}} Q = \sum_{i=1}^{3} w_i \, f_i(\mathbf{u}) - \sum_{j=1}^{5} r_j \int_{0}^{t_f} g_j(t) \, dt, \qquad \sum_{i=1}^{k} w_i = 1 \tag{14}$$

where: w_i – weight coefficients, r_j – penalty parameters.

The single-objective functions $f_i(\mathbf{u})$ are normalized in the range from 0 to 1. The weights w_j was connected with the importance of the he separate single-objective criterion $f_i(\mathbf{u})$. In this study w_j were chosen in the following way $w_j = [0.5, 0.3, 0.2]$, where basic weight in the general criterion was interpreted as the process productiveness Eq. (10). The second importance is the substrate utilization degree Eq. (11) and the last one is the mass-transfer in the bioreactor.

The optimal decision $\mathbf{u}^*$ maximizing the general utility function (14) was found by using of a fuzzy sets theory method.

Fuzzy optimal control

The fuzzy sets theory (FTS) allows a possibility to be developed a "*flexible*" model [15], that reflects in more details about criterion possible values and control variables of the developed model. The developed model was evaluated as the most acceptable one. As admissible, but with a less degree of acceptability were evaluated some diversions of the developed model. This was presented by a fuzzy set with a membership function of the following type:

$$v_i = \frac{1}{1 + \varepsilon_i^2} \tag{15}$$

where: ε_i – deviation of the basic model, $i = 1, \ldots, N$ – number of equations in the main model.

The fuzzy criterion was formulated as follows: "*The optimum criterion $Q(\mathbf{u})$ to be possibly higher*" and it was presented by the following membership function:

$$v_0(Q) = \begin{cases} 0; & Q < Q_L \\ \dfrac{Q - Q_L}{Q_U - Q_L}; & Q_L \le Q \le Q_U \\ 1; & Q > Q_U \end{cases} \tag{16}$$

where Q_L and Q_U were lower and upper values for criterion Q.

The following optimization problem in the class of the fuzzy mathematical programming problems can be formulated:

$$Q \cong \underset{\mathbf{u}}{\mathrm{m\tilde{a}x}}\, Q \tag{17}$$

where "$m\tilde{a}x$" means *"in possibility maximum"*; "$\cong$" means *"is come into view approximately in following relation"*.

For determination of this problem, an approach generalizing the Bellman-Zadeh's method [1] was used. The fuzzy set of the solution was presented with a membership function $v_D(\mathbf{u})$, whish was conjunction of the membership functions of the fuzzy set of the criterion $v_0(\mathbf{u})$ and the model $v_i(\mathbf{u})$:

$$v_D = (1 - \gamma)\prod_{i=0}^{N} v_i^{\theta_i} + \gamma\left(1 - \prod_{i=0}^{N}(1 - v_i)^{\theta_i}\right) \tag{18}$$

where: γ – parameter characterized the compensation degree;
  θ_i – the weights of v_i, $(i = 0, 1, …, N)$.

The solution was obtained by using common *defuzzification* method BADD [1, 5]:

$$\mathbf{u}^* = \sum_{i=1}^{q} \lambda_i \mathbf{u}_i, \quad \lambda_i = \frac{v_{D_i}^{\theta_i}(\mathbf{u})}{\sum_{j=1}^{p} v_{D_j}^{\theta_i}(\mathbf{u})}, \quad i = 1, ..., q; \quad j = 1, 2, ..., p; \quad p = q^{n_c} \tag{19}$$

where: q – number of discrete values of the vector $\mathbf{u}$, n_c – number of control variables.

The generalized fuzzy algorithm scheme can be described as follows:

BEGIN

1. Input: n_c – number of control variables, m – number of criterion;
     w_j – weight of each criterion, $(j = 1, m)$;
     K – number of time partitions;
     q – number of discrete values of vector $\mathbf{u}$;
2. Computing criterion Q_B before optimization from (14);
3. Computing low and upper values for criterion: $Q_L = 0.80Q_B$ and $Q_U = 1.20Q_B$;
4. Computing discrete values of each control variable $\mathbf{u}$;
5. Computing of deviations ε_i from the basic model;
6. Computing of membership functions of the model v_i, $(i = 1, 2, …, N)$ from (15);
7. Computing of single-objective function Q from (17);

8. Computing of membership function of the criterion v_0 from (16);
9. Computing of membership function of the decision v_D from (18);
10. Obtaining of solution $\mathbf{u}^*$ using *defuzzification* operator   from (19);
11. Returns optimal values of each control variables $\mathbf{u}^*$;
12. Computing model after fuzzy optimal control;
13. Print results: model before and after optimal control; single-objective functions f_i and general function Q; each control variable in the time partitions.

END

All computations were performed on a Pentium IV 1.8 GHz computer using a Windows XP operating system. The Fuzzy algorithm was written on a FORTRAN programming language version 5.0.

Results and discussion

The discrete values of each control variable is presented in Table 1, ($j = 1, 2, …, 12$).

Table 1

$F(j)\text{x}10^3$, [l·h^{-1}]	4	8	13	17	21	25	29	33	38	42	46	50
$n(j)$, [rpm]	633	667	700	733	767	800	833	867	900	933	967	1000
$Q(j)$, [l·h^{-1}]	65	70	75	80	85	90	95	100	105	110	115	120

The optimal values of feed flow rate, agitation speed, and gas flow rate are shown in Fig. 1 to Fig. 3. The biomass and substrate profiles are shown in Fig. 4. The oxygen profile is presented in Fig. 5.
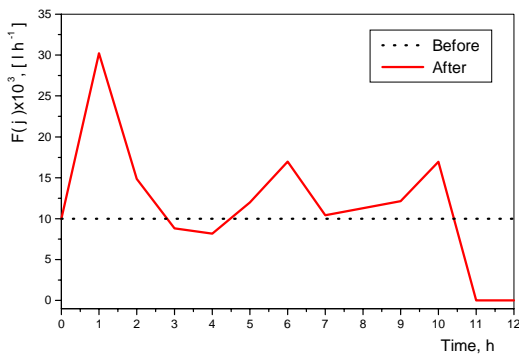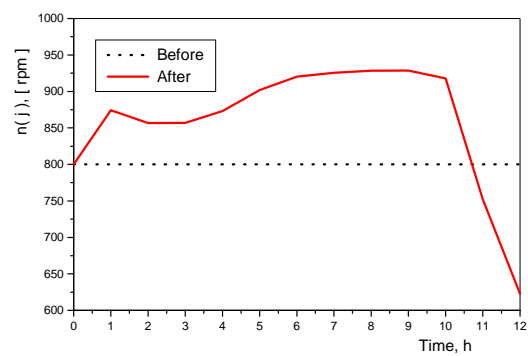

Fig. 1 Optimal feed flow rate profile

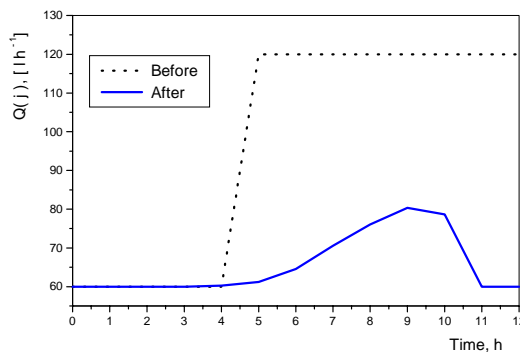Fig. 2 Optimal agitation speed profile

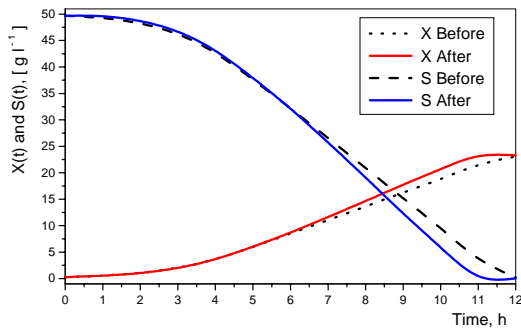Fig. 3 Optimal gas flow rate profile

Fig. 4 Concentration profiles
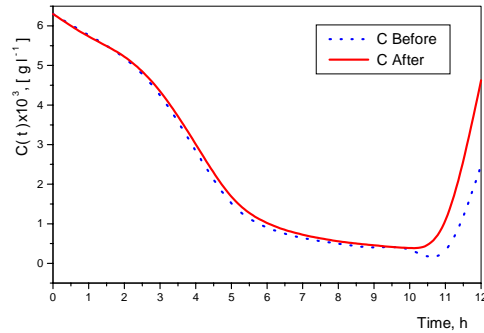of biomass and substrate

Fig. 5 Concentration profiles of
dissolved oxygen

From Fig. 1 to Fig. 3 is shown a vastly change of the control variable as a function time. It is especially true about the feeding flow rate and gas flow rate.

When the gas flow rate was included as a control variable it should be noted that the gas consumption is considerable less. This is especially important in industrial scale, where the energy consumptions in fermentation step have determined product cost.

From Fig. 4, one can observe that the biomass concentration has increased slightly. This fact is well known from the biochemical engineering point of view and can be interpreted like stationary biomass growth phase where the nutrients are exhausted. Analyzing the presented results in this figure one may conclude that there was better substrate consumption under the optimal control.

The obtained optimal profile of dissolved oxygen concentration versus time has shown that the agitation cost is minimized (Fig. 5). This fact should be analyzed with caution and the oxygen values should be compared with the critical oxygen concentration values below which a dissolved oxygen limitation takes place.

Conclusions

1. The obtained results from the study have shown that multiple-objective optimization is more complex approach minimizing the risk in the procedure of making of decisions and maximizing the formulated objective.
2. The used method based on the fuzzy sets theory has allowed a direct search of the fuzzy optimization problem and gives non-stochastic indeterminateness neglect in the tradition methods. Hence, the general defects of the numerical decision have been avoided. A disadvantage of the method is that presents the unknowns in discrete form, which make the method less successful for large-scale problems.
3. The obtained optimal profiles of the feed flow rate, agitation speed and gas flow rate and received results after the theoretical optimal control have shown clearly practical applicability of the used techniques, in particular, for maximization of process productivity.

Acknowledgements

References

1. Angelov P. (1994). Generalized Approach to Fuzzy Optimization, Int. J. of Intelligent Systems, 9(3), 261-268.
2. Angelov P., M. Petrov, S. Tzonkov (1996). An Approach to Optimal Control of Biotechnological Processes Based on Soft Computing, Int. Conference "Automatic and Informatics", October 9-11, Sofia, Bulgaria, 252-255.
3. Chen Y., F. Wang (2003). Crisp and Fuzzy Optimization of a Fed-batch Fermentation for Ethanol Production, Industrial and Engineering Chemistry Research, 42, 6843-6850.
4. Choi K., D. Butala (1991). An Experimental Study of Multiobjective Dynamic Optimization of a Semibatch Copolymerization Process, Polym. Eng. Sci., 31, 353.
5. Filev D., R. Yager (1991). A Generalized Defuzzification Method via Bad Distribution, Int. J. of Intelligent Systems, 6, 687-697.
6. Fu P., J. Barford (1993). Non-Singular Optimal Control for Fed-batch Fermentation Processes with a Differential-Algebraic System Model, J. Proc. Control, 3, 211-219.
7. Messac A., S. Gupta, B. Akbulut (1996). Linear Physical Programming: A New Approach to Multiple Objective Optimization, Transactions on Operational Research, 8, 39-59.
8. Petrov M. (2006). A Combined Algorithm for Fuzzy Optimization and Optimal Control of Whey Fermentation in Stirred Tank Bioreactors, Int. Symposium "Bioprocess Systems-BioPS'06", October 24-25, Sofia, Bulgaria, I.31-I.38.
9. Sendín O., J. Vera, T. Nestor (2006). Model Based Optimization of Biochemical Systems using Multiple Objectives: A Comparison of Several Solution Strategies, Mathematical and Computer Modelling of Dynamical Systems, 12(5), 469-487.
10. Simov I., E. Simova, M. Beshkova (2006). Impact of Two Starter Cultures on Proteolysis in Kashkaval Cheese, Word Journal of Microbiology and Biotechnology, 22(2), 147-156.
11. Tonon F., A. Bernardini (1999). Multiobjective Optimization of Uncertain Structures Through Fuzzy Sets and Random Set Theory, Computer-aided Civil and Infrastructure Engineering, 14, 119-140.
12. Vera J. at al. (2003). Multicriteria Optimization of Biochemical Systems by Linear Programming. Application to the Ethanol Production by *Saccharomyces cerevisiae*, Biotechnology and Bioengineering, 83(3), 335-343.
13. Wang F. et al. (1998). Fuzzy-Decision-Making Problems of Fuel Ethanol Production using a Genetically Engineered Yeast, Industrial and Engineering Chemistry Research, 37, 3434-3443.
14. Wang F. S., K. J. Lin (2003). Performance Analysis and Fuzzy Optimisation of a Two-Stage Fermentation Process with Cell Recycling Including an Extractor for Lactic Acid Production, Chemical Engineering Science, 58, 3753-3763.
15. Zadeh L. (1999). Fuzzy Sets as a Basis for a Theory of Possibility, Fuzzy Sets and Systems, 100, 9-34.
16. Zhou Y. H., N. J. Titchener-Hooker (2003). The Application of a Pareto Optimisation Method in the Design of an Integrated Bioprocess, Bioprocess and Biosystems Engineering, 1-18.