

CULTIVATION PROCESS OPTIMIZATION BASED ON GENETIC ALGORITHMS

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Abstract

*Various approaches have been used to describe nonlinear behaviour of bioprocesses. Some of them are directly based on the divide-and-conquer strategy. These, so-called local approaches can often give a simplified and transparent nonlinear model or optimal control representation. The cultivation process can be divided into several functional states (FS) by considering the cell metabolism in more detail. The maintenance of the appropriate FS could ensure an optimal run of the process. In this paper a feed rate profile that leads to defined FS is synthesized. For the profile synthesis a genetic algorithm (GA) is developed and applied using four objective functions. Nowadays the most common direct methods used for global optimization are GA. The GA are directed random search techniques, based on the **mechanisms** of natural selection and genetics, which can find a global optimal solution in complex multidimensional search spaces. The obtained results have presented the ability of GA to generate an optimal feed rate profile based on defined rules. The GA generates feed rate profile, that for a short time leads the process to the FS, determined as optimal, and maintains this state during the process. As a result a high final biomass concentration is achieved under optimal conditions for amount and assimilation of the substrate.*

Key words: Cultivation Process, Functional States, Feed Rate Profile, Genetic Algorithm, Optimal Control

1. INTRODUCTION

When controllable microbial synthesis is realized it should be taken into account the fact, that the control is carried out in the biological (live) system and that the microorganisms possess the features for “memory” of culture, self-control, self-production etc. Moreover, cultivation processes are proliferating open systems (capable of self-reproduction) with steady passing through themselves flows of matter and energy.

The common modelling approach that has been extensively used when such complex processes are modelled is the development of an overall nonlinear model that performs satisfactorily through the entire operating range. As a result a global process model is synthesized. Unfortunately, this approach has a lot of disadvantages. As much more detailed description of the process is aimed as more complex model will be obtained. Complex global models are characterized with a great number of parameters, which complicate the model identification, simulation and control synthesis. Moreover, a global model is not able to describe the metabolic changes during the entire operating range. Another particularity that the global models do not consider is the parameter non-stationary. In order to avoid mentioned above disadvantages an alternative approach for model development based on multiple-model framework is applied. Using this approach, complicated problems are decomposed into

subproblems that can be solved independently. Then the individual solutions of the decomposed problems lead to a global solution of the complex problem [7].

The multiple-model based approach is an appropriate tool for monitoring and control of cultivation processes [6, 7, 9, 14, 15, 20]. The decomposition into operating regimes could be considered as the most successful methodology. Thus, the process is decomposed into different stages – functional states [9], corresponding to the stages, through which the process pass. Using this methodology more precisely description of the process performance is achieved based on relatively simpler models. This methodology allows partial transparency of process knowledge to the modelling procedure.

It should be noted that the cultivation process could be only in one functional state at a given time. However, a certain functional state can appear in the process more than once during one run. Depending on the goals of cultivation process only some functional states can appear. Not all possible functional states are desired for the optimal process run. For example, if the *E. coli* cultivation process enter in the second acetate production state [12, 13] and stays in this state it will result in growth inhibition even in stopping the culture growth. Therefore, it is necessary for optimal cultivation process maintaining of the system in the definite functional states to be ensured. An optimal state of microorganisms' culture for biosynthesis of the desired product can be maintained by using appropriate feed rate profiles. The appearance of the each functional state could be controlled trough the feed rate profile of the cultivation process. Thus, an optimal condition for the culture growth will be ensured and a productivity increase will be achieved.

Optimization of fed-batch cultivation processes has been a topic of research for many years. Control opportunities in fed-batch operated cultivations have been reviewed in detail in a number of articles. Currently, the feed rate optimization problem is commonly solved by mathematical model based optimization methods. If an accurate model of the system is available, optimization procedures can be used to calculate the feeding strategy [3, 16, 17]. However, cultivation process properties make difficult the control with traditional techniques. For simple mathematical models, the problem can be solved analytically, from the Hamiltonian function, by applying the minimum principle of Pontryagin [18, 19]. Besides having a problem of singular control, those methodologies become too complex when the number of state variables increases. As an alternative overcoming these difficulties the global optimization methods are used.

Nowadays the most common direct methods used for global optimization are evolutionary algorithms such as genetic algorithms (GA). GA are direct random search techniques, based on the mechanisms of natural selection and genetics, which can find a global optimal solution in complex multi-dimensional search spaces. GA have been used extensively in solving many optimization-searching problems [1, 2, 4, 8, 11, 10]. Compared with traditional optimization methods, GA simultaneously evaluates many points in the parameter space. It is more probable to converge towards the global solution. A genetic algorithm does not assume that the space is differentiable or continuous and can also iterate many times on each received data. A GA requires only information concerning the quality of the solution produced by each parameter set (objective function value information). This characteristic differs from optimization methods that require derivative information or, worse yet, complete knowledge of the problem structure and parameters. Since GA do not demand such problem-specific information, they are more flexible than most search methods. Also GA do not require linearity in the parameters which is needed in iterative searching optimization techniques. Genetic algorithms can solve hard problems, they are noise tolerant, easy to interface to existing simulation models, and easy to hybridize.

Here is introduced a synthesis of optimal feed rate profiles using GA. The calculated feeding trajectory leads the process to the defined desired functional state and keeps the state to the end of the process. A

mathematical model of *E. coli* fed-batch cultivation is studied [9]. The bacterium *E. coli* is the microorganism chosen for the production of the majority of the valuable biopharmaceuticals. *E. coli* usually grows under fed-batch mode due to the effect of acetic acid, which is produced when glucose is above certain concentrations. Therefore, it is important to control the process in a way to avoid the inhibition effect of the acetate.

2. FUNCTIONAL STATES ANALYSIS FOR E. COLI CULTIVATION PROCESS

2.1. Mathematical model of *E. coli* cultivation process

The concept of functional state modelling is successfully applied for description of several fed-batch cultivations of *E. coli* [9]. The following assumptions are made in the local models' development:

- The bioreactor is completely mixed.
- The suspension viscosity in the reactor remains constant during the experiment.
- Variations in the growth rate and in substrate consumption do not significantly change the elemental composition of biomass, thus balanced growth conditions are only assumed.
- Parameters, e.g. pH and temperature, are controlled to certain constant values.

The mathematical model, based on the mass balance of the components (biomass, glucose, acetate, dissolved oxygen and carbon dioxide), is presented by the following differential equations:

$$\frac{dX}{dt} = \mu X - \frac{F}{V} X \quad (1)$$

$$\frac{dS}{dt} = -q_S X + \frac{F}{V} (S_{in} - S) \quad (2)$$

$$\frac{dA}{dt} = q_A X - \frac{F}{V} A \quad (3)$$

$$\frac{dO_2}{dt} = -q_{O_2} X + k_L^{O_2} a(O_2^* - O_2) - \frac{F}{V} O_2 \quad (4)$$

$$\frac{dCO_2}{dt} = q_{CO_2} X + k_L^{CO_2} a(CO_2^* - CO_2) - \frac{F}{V} CO_2 \quad (5)$$

$$\frac{dV}{dt} = F \quad (6)$$

where: X is biomass concentration, [g l⁻¹]; S – glucose concentration, [g l⁻¹]; A – acetate concentration, [g l⁻¹]; DO – dissolved oxygen tension, [%]; CO_2 – carbon dioxide tension, [%]; DO^* – saturation concentration of dissolved oxygen tension, [%]; CO_2^* – saturation concentration of carbon dioxide tension, [%]; F – feed rate, [l h⁻¹]; V – bioreactor volume, [l]; S_{in} – glucose concentration of feed, [g l⁻¹]; μ – specific rate of biomass growth, [gg⁻¹h⁻¹]; $Y_{S/X}$ – yield coefficient for biomass, [gg⁻¹]; $Y_{A/X}$ – yield coefficient for acetate, [gg⁻¹]; $Y_{DO/X}$ – yield coefficient for dissolved oxygen tension, [gg⁻¹]; $Y_{CO_2/X}$ –

yield coefficient for carbon dioxide, $[\text{gg}^{-1}]$; $k_L^{DO} a$ – volumetric oxygen transfer coefficient, $[\text{h}^{-1}]$; $k_L^{CO_2} a$ – volumetric carbon dioxide transfer coefficient, $[\text{h}^{-1}]$.

Structures of specific rate functions μ , q_s , q_A , q_{O_2} and q_{CO_2} vary in connection with the recognized functional states. The values of substrate and dissolved oxygen critical levels are [9]:

$$S_{crit} = 0.1 \text{ g l}^{-1} \text{ and } O_{2crit} = 20\%. \quad (7)$$

The whole bacteria growth process can be divided into five FS in batch and fed-batch cultures [9]:

- *First acetate production state (FS I)*
- *Mixed oxidative state (FS II)*
- *Complete sugar oxidative state (FS III)*
- *Acetate consumption state (FS IV)*
- *Second acetate production state (FS V)*

Each functional state is characterized with specific biochemical processes depending on current cultivation condition. Considering an *E. coli* fed-batch cultivation process these particularities are described in [12]. A briefly description is presented below.

First acetate production state (FS I)

The *E. coli* growth process is defined to be in this state when the S is above the critical level (S_{crit}), there is sufficient dissolved oxygen (pO_2) and acetate (A) is produced. This acetate excretion is the result of a metabolic “overflow” mechanism occurring when the carbon (glucose) flux into the central metabolic pathways exceeds the biosynthetic demands and the energy generation capacity of the cell. This by-product induces a stress response and reduces the production of recombinant proteins.

Mixed oxidative state (FS II)

The process enters into this state when the sugar concentration decreases and becomes equal to or below the critical level, and there is sufficient dissolved oxygen. Both sugar and produced acetate are cometabolised through the oxidative pathways in the state.

Complete sugar oxidative state (FS III)

The process is defined to be in this state when there is no acetate available, the sugar concentration is not higher than the critical level and the dissolved oxygen is above its critical level (pO_{2crit}). In this state, sugar is completely oxidized to water and carbon dioxide, and there is no acetate formation. Continuing from *FS II* we assume that acetate is depleted faster by the bacteria or removed from the media and the only carbon source left in the media is glucose.

Acetate consumption state (FS IV)

The process is defined to be in this state when acetate is available but there is no sugar in the broth, and the dissolved oxygen concentration is above the critical level. Acetate is the only carbon source for *E. coli* growth. This state can start from *FS I* when the glucose in the media is depleted and the only carbon source is acetate. Acetate is completely metabolized to water and carbon dioxide.

Second acetate production state (FS V)

The conditions for this state are both sugar and dissolved oxygen concentrations to be below the corresponding critical levels. When the dissolved oxygen becomes the limiting factor for *E. coli* growth, acetate is produced. This acetate will be used after that for consumption by the cell if dissolved oxygen concentration becomes above the critical minimum.

An *E. coli* growth process switches from one functional state to another when the metabolic conditions are changed. The functional state diagram of this process can be illustrated with Fig. 1. The *FS IV* is not considered here because that functional state appears only in batch cultivation processes.

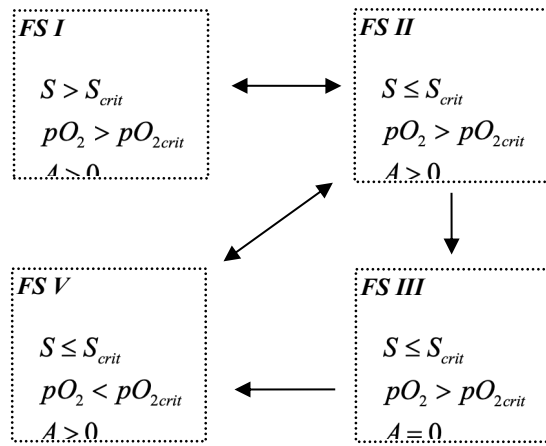


Fig. 1. Interrelationships and rules for recognition of functional states

In principle *FS I* can appear in all types of *E. coli* growth processes (batch, fed-batch and continuous cultivations). With the decreasing level of the glucose concentration the process switches from *FS I* to *FS II*. At this point the process can be easily switched back to *FS I* with the addition of glucose or it can continue on different pathway. If the level of glucose is kept close to the critical point and the level of acetate is low, the acetate will be depleted fast. This will set the process to *FS III*. From there the system can switch to *FS V* where the level of dissolved oxygen and glucose are below their critical points. Starting directly from *FS II* reducing the levels of dissolved oxygen and glucose the process can switch to *FS V* and vice versa using the same pathway.

Through the analysis of the functional state it could be considered as desired functional states *FS II* and *FS III*. In the *FS II* there is sufficient dissolved oxygen and both sugar and acetate are cometabolised. In the *FS III* there is no acetate formation and sugar is completely oxidized to water and carbon dioxide. In both functional states there are no conditions for process inhibition.

The rules for *FS II* and *FS III* recognition are, respectively [9]:

$$\begin{array}{ll}
 S \leq S_{crit}, & S \leq S_{crit} \\
 pO_2 > pO_{2crit} \text{ and } & pO_2 > pO_{2crit} \\
 A > 0 & A = 0
 \end{array} \quad (8)$$

To simplify the recognition rules (9) they could be integrate into one rule, without consideration of acetate concentration:

$$S \leq S_{crit}, pO_2 > pO_{2crit} \quad (9)$$

The local model for considered functional states is presented as:

$$\begin{aligned} \frac{dX}{dt} &= \mu_{max} \frac{S}{k_S + S} X + \mu_{maxA} \frac{A}{k_A + A} X - \frac{F}{V} X \\ \frac{dS}{dt} &= -\frac{1}{Y_{S/X}} \mu_{max} \frac{S}{k_S + S} X + (S_{in} - S) \frac{F}{V} \\ \frac{dA}{dt} &= -\frac{1}{Y_{A/X}} \mu_{maxA} \frac{A}{k_A + A} X - \frac{F}{V} A \\ \frac{dO_2}{dt} &= -\left(\frac{1}{Y_{O_2/S}} \mu_{max} \frac{S}{k_S + S} + \frac{1}{Y_{O_2/A}} \mu_{maxA} \frac{A}{k_A + A}\right) X + k_L^{O_2} a(O_2^* - O_2) - \frac{F}{V} O_2 \\ \frac{dCO_2}{dt} &= -\left(\frac{1}{Y_{CO_2/S}} \mu_{max} \frac{S}{k_S + S} + \frac{1}{Y_{CO_2/A}} \mu_{maxA} \frac{A}{k_A + A}\right) X + k_L^{CO_2} a(CO_2^* - CO_2) - \frac{F}{V} CO_2 \\ \frac{dV}{dt} &= F \end{aligned} \quad (10)$$

Using the model (10) the genetic algorithm will calculate the feed rate profile following the rule (9). Resulting trajectory is defined as optimal – in the system will be sufficiently dissolved oxygen, the substrate concentration will be kept below the critical value and there will not be critical accumulation of acetate during the cultivation process.

3. GENETIC ALGORITHM APPLICATION FOR OPTIMAL FEED RATE SYNTESIS

Simple genetic algorithms are guided largely by the mechanisms of three operators: reproduction, crossover and mutation [5]. To derive a solution to a problem, the simple genetic algorithms initialize a single population of n randomly encoded chromosomes (individuals). Each chromosome corresponds to one different objective function value. The objective functions (cost values) of generated population are then evaluated. In the next step individuals represented by their associated cost are ranked and the corresponding individual fitness is received. The selection algorithm chooses individuals for reproduction on the basis of their relative fitness. Thus solutions from one population are taken and used to form a new population.

Outline of the proposed genetic algorithm could be presented as:

1. **[Start]** Generate random population of n chromosomes – n feed rate profiles
2. **[Fitness]** Evaluate the fitness $f(x)$ of each chromosome x in the population
3. **[New population]** Create a new population by repeating following steps until the new population is complete
 - 3.1. **[Selection]** Select two parent chromosomes from a population according to their fitness
 - 3.2. **[Crossover]** With a crossover probability cross over the parents to form new offspring
 - 3.3. **[Mutation]** With a mutation probability mutate new offspring at each locus
 - 3.4. **[Accepting]** Place new offspring in the new population

4. **[Replace]** Use new generated population for a further run of the algorithm
5. **[Test]** If the end condition is satisfied, stop, and return the best solution in current population
6. **[Loop]** Go to step 2

Representation of chromosomes is a critical part of GA application. Here, each chromosome of the population represents a feed rate profile as a sequence of feed rate values. The simplest way to represent it was by using a piecewise approximation of the feed rate profile. The profile is divided into equal intervals of 20 minutes and the feed rate values at the breakpoints are registered. The sequence of numbers obtained is considered a chromosome and each gene represented the feed rate after 20 minutes. Each gene is coded in feed rate range $0 - 0.05 \text{ lh}^{-1}$.

An evaluation function plays a role similar to that which the environment pays in natural evolution and it rates chromosome in terms of fitness. The objective functions utilized here are: sum of the absolute error over the cultivation time; sum of the time multiplied by absolute error; sum of the square time multiplied by absolute error.

In the GA, there are many operators, functions, parameters and settings that can be implemented differently in various problems. The GA parameters significantly affect the speed of convergence to the near optimal solution, and the accuracy of the solution itself. Here is also investigated the effect of the different GA parameters on the outcome of the GA enhanced simulation.

4. RESULTS AND ANALYSIS

4.1. Tuning the GA performance

Since GA are stochastic, their performance usually varies from generation to generation. Extensive simulation tests have been conducted on the GA to test the effectiveness of the algorithm. The first set of experiments was carried out in order to find the best set of genetic operators to tackle with the feed rate optimization problem. Each run of the GA is stopped after 100 iterations and the results are given in terms of the mean of 25 runs, with the associated 95% confidence intervals.

Moreover, there were performed a lot of tests to choose the appropriate GA parameters for the considered here problem. The performed tests held most elements of GA constant while one element was changed.

Summary of the performed tests:

A binary 20 bit encoding is considered. Binary representation is the most common one, mainly because of its relative simplicity. The best known selection mechanism, roulette wheel selection, is used in the proposed GA.

The genetic operators used in this GA are namely, reproduction, crossover and mutation. Offspring are normally different from parents due to the genetic information exchange process, e.g. chromosome crossover. However, in GA, the reproduction process is merely a simple coping activity which passes the parent's genetic information to the offspring. The reproduction process usually acts as a complementary process of crossover activity and the offspring are either created by reproduction or crossover.

Crossover is an extremely important component of the GA as it is responsible for searching through the solution space. Crossover can be quite complicated and depends (as well as the technique of mutation) mainly on the encoding of chromosomes. Here, double point crossover is employed. After a crossover is performed, mutation takes place. Mutation reintroduces diversity into the population. In

the accepted encoding here a bit inversion mutation is used. This prevents the solution from converging to some local optimal solutions; thereby the global optimal solution can be obtained.

Particularly important parameters of GA are the population size (number of individuals) and number of generations. If there is too low number of chromosomes, GA has a few possibilities to perform crossover and only a small part of search space is explored. Small populations cause premature convergence. Large population sizes take a longer time to run but converge faster than the smaller populations. A number of individuals of 150 is used as a compromise. The higher crossover rates lead to better relative fitness function values. A crossover rate of 0.75 is used. Zero mutation probability causes premature convergence to a suboptimal value. High mutation probabilities, on the other hand, cause fluctuations and disturb convergence. Generally, the mutation probability should be high enough to expand the search space, but low enough to bring about convergence. A mutation probability of 0.01% is used as a compromise.

Based on these results, chosen GA operators and parameters for the considered here problem are summarized in Table 1.

Table 1. Genetic algorithm elements

Operator	Type	Parameter	Value
encoding	binary	crossover rate	0.75
crossover	double point	mutation rate	0.01
mutation	bit inversion	encoding precision	20
selection	roulette wheel selection	number of individuals	150
fitness function	linear ranking	number of generations	150

4.2. Feed rate profile synthesis

Due to the stochastic nature of the GA for each defined objective function at least 30 executions of the algorithm are performed. More than 200 simulations are carried out. The results show that the algorithm produce the same results with more than 80% coincidence. For the **statistical correctness** of the results average values of the outcomes (dynamics of the main state variables – biomass, substrate, acetate, dissolved oxygen and carbon dioxide, as well as the synthesized feed rate profile) at a certain objective function are calculated. In the next figures some of the significant and qualitative results are presented.

The resulting feed rate profile using the objective function – sum of the absolute error over the cultivation time, as well as dynamics of the main state variables are presented on the Fig. 2.

The substrate concentration is kept below to the critical concentration with a small period of excess substrate in the broth (between 8 – 8.20 h of cultivation). The calculated feed rate profile keeps the dissolved oxygen tension above considered critical value of 20%. There is a period with data below critical value, but it is for a very short time and after that the dissolved oxygen tension is normalized. Thus, the desired functional state is maintained and optimal growth conditions are ensured. The cell concentration increases over the cultivation period and achieves the value 4.70 g l⁻¹. The acetate concentration is in the acceptable range and does not cause an inhibition effect on the cell growth.

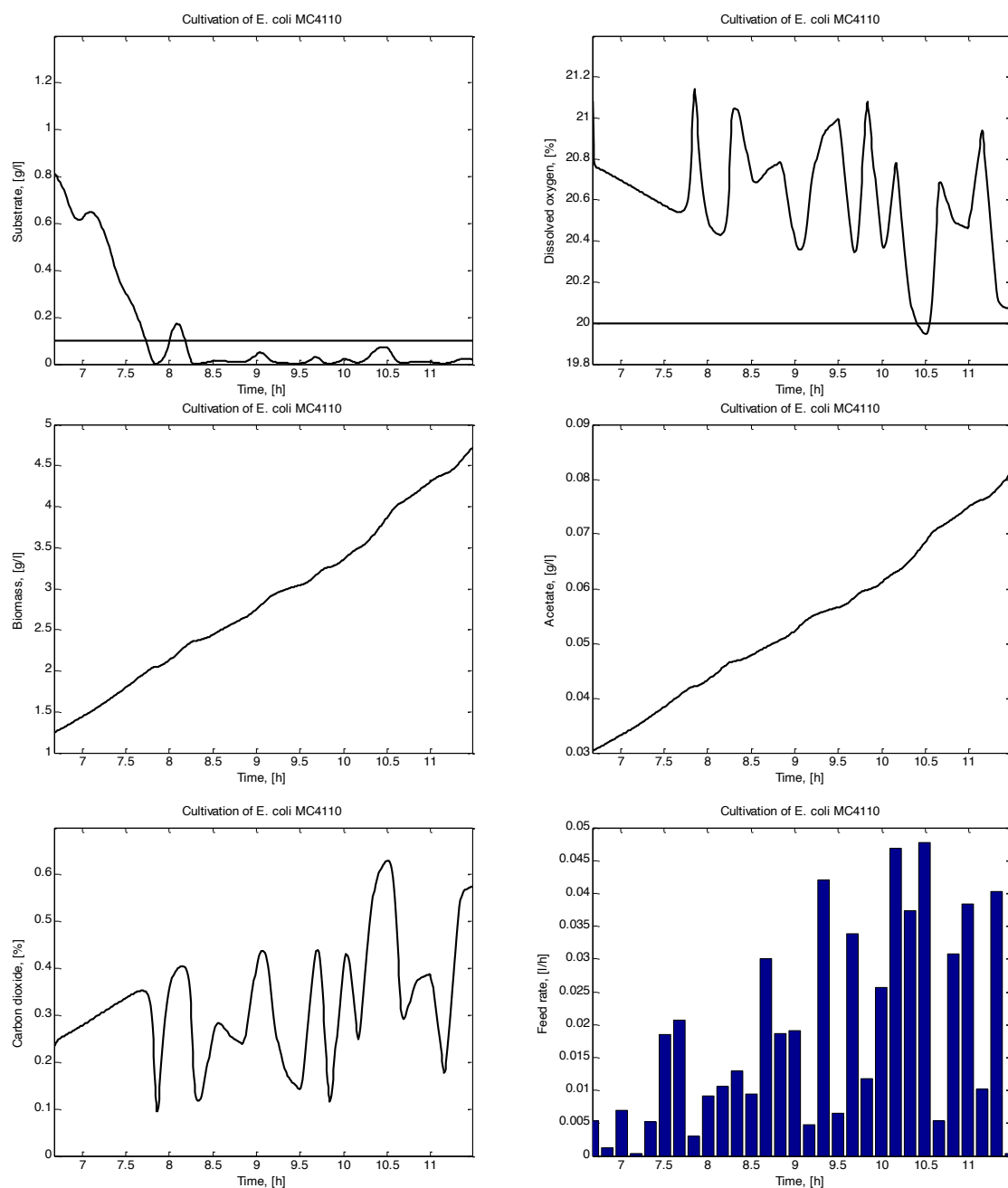


Fig. 2. Dynamics of the main process variables and optimal feed-rate profile – average result
objective function – sum of the absolute error

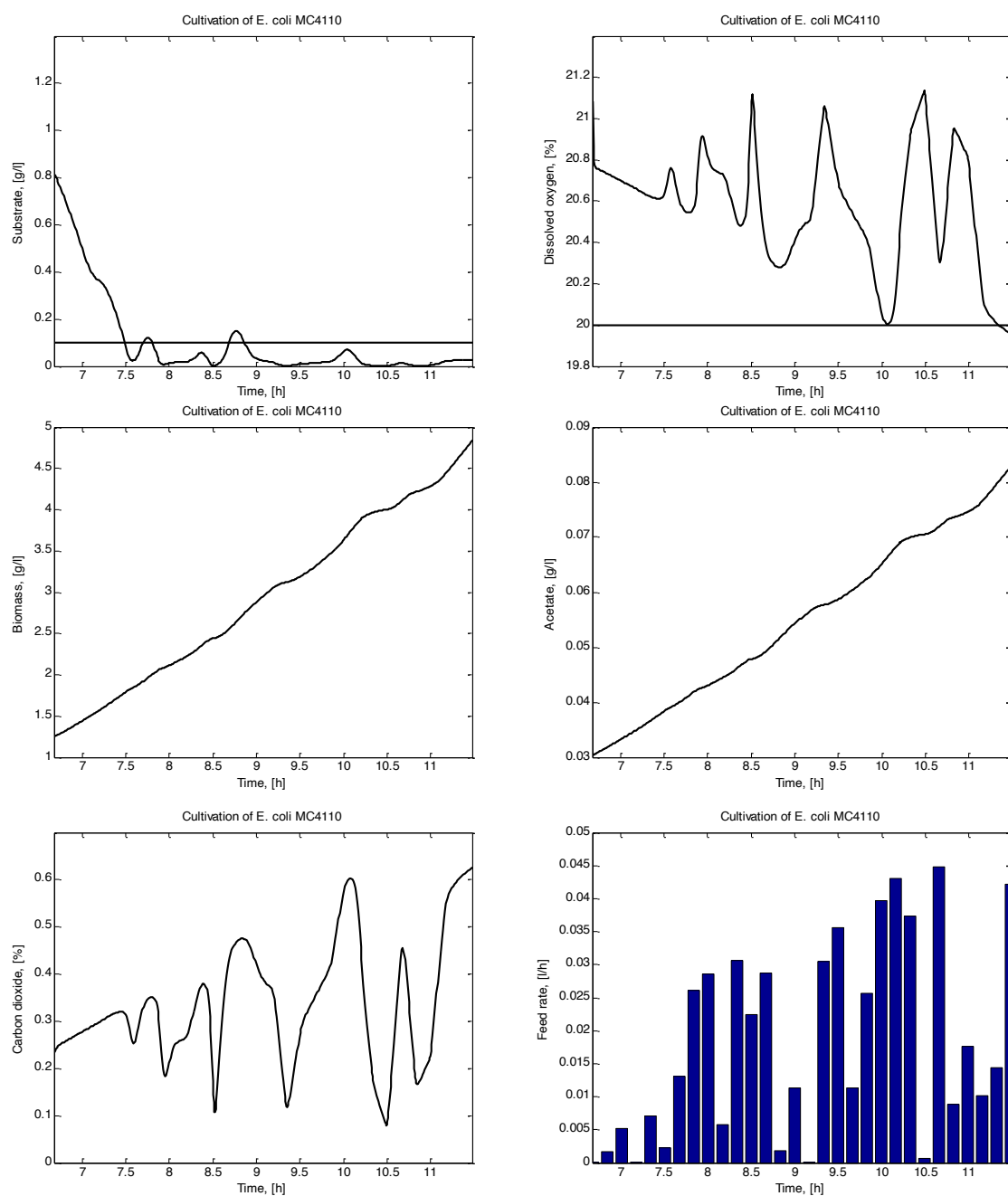


Fig. 3. Dynamics of the main process variables and optimal feed-rate profile – one of the 30 runs

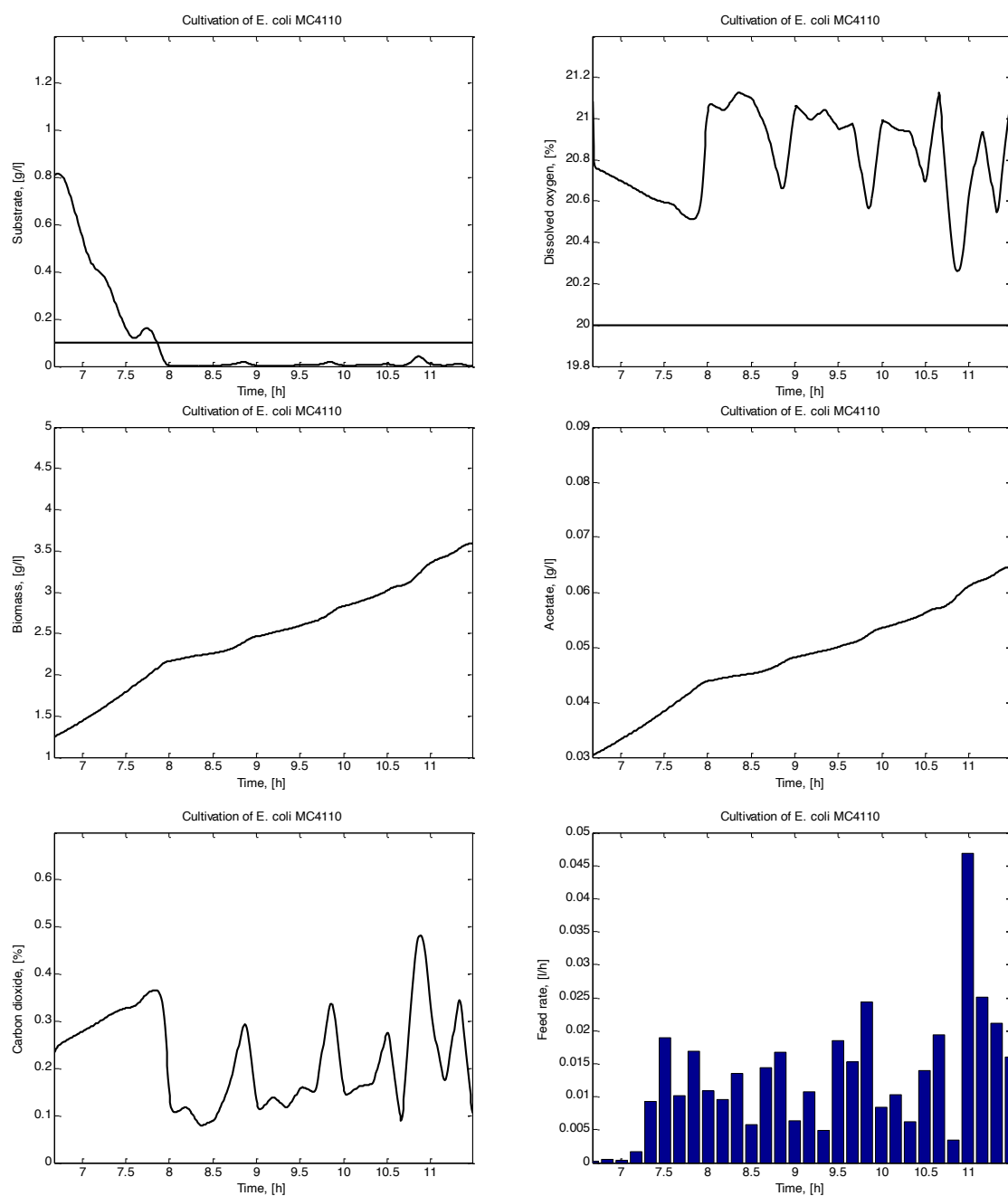


Fig. 4. Dynamics of the main process variables and optimal feed-rate profile – average result
objective function – sum of the absolute error

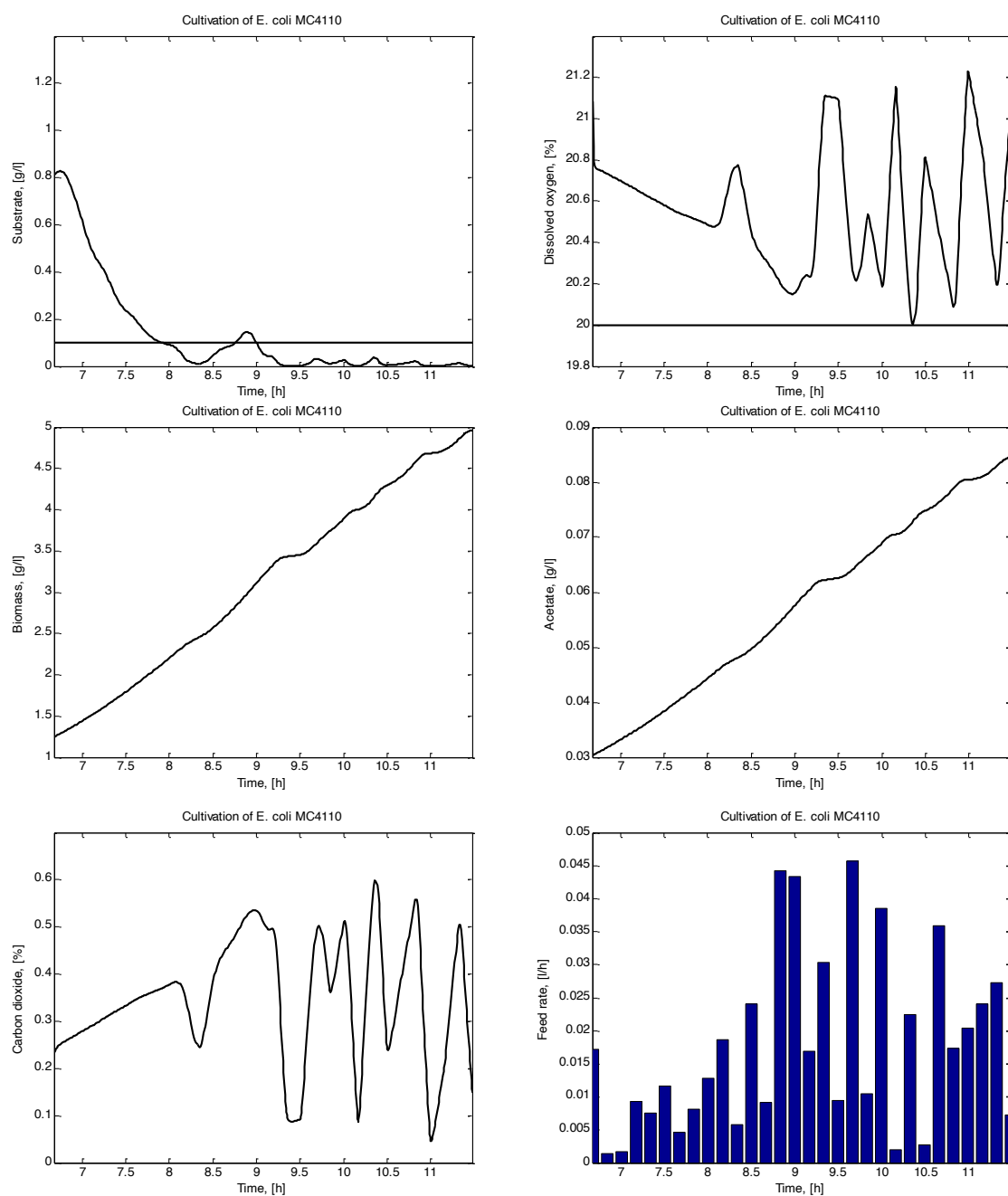


Fig. 5. Dynamics of the main process variables and optimal feed-rate profile – average result objective function – sum of the time multiplied by absolute error

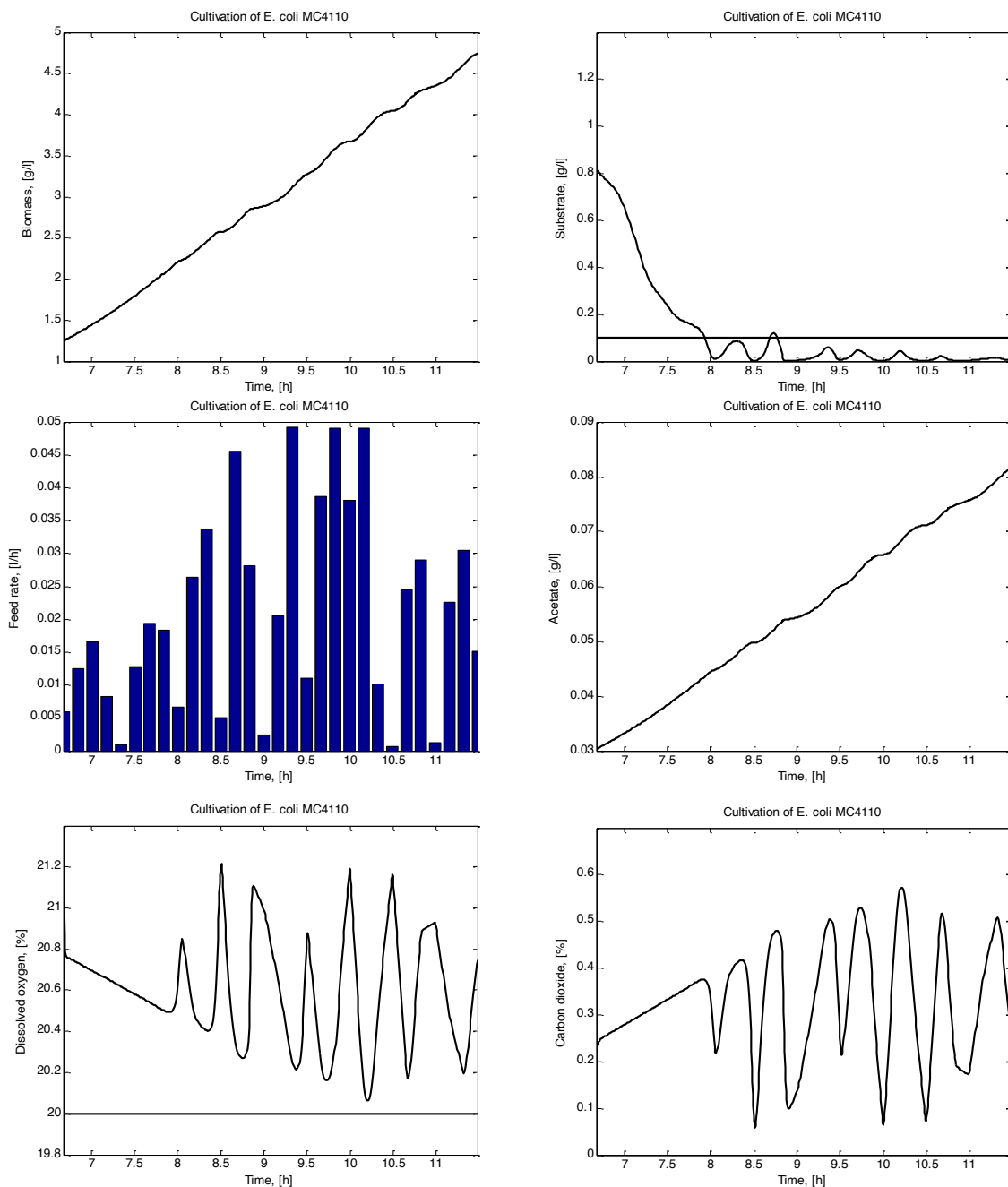


Fig. 6. Dynamics of the main process variables and optimal feed-rate profile – average result objective function – sum of the square time multiplied by absolute error

The result from one of the 30 runs that ensures desired functional state for a shorter time period is depicted on Fig. 3. The substrate concentration reaches the critical concentration at the 7.5 h of cultivation. After the 7.5 h the substrate concentration is kept below to the critical concentration with

two period of excess substrate (around 7.75 h and 8.75 h of cultivation). The final cell concentration is 4.87 gl^{-1} . The presented result is similar to the above considered one.

If it is assumed lower value of the critical substrate concentration, for example $S_{crit} = 0.05 \text{ gl}^{-1}$, the obtained substrate dynamics leads to decrease of the final biomass concentration. The results are shown on Fig. 4.

If it is assumed higher value of the critical substrate concentration, for example $S_{crit} = 0.2 \text{ gl}^{-1}$, there is no significant increase of the final biomass concentration. These experiments confirm the considered critical substrate concentration of 0.1 gl^{-1} as appropriate for the regarded cultivation process.

The resulting feed rate profile using the objective function – sum of the time multiplied by absolute error over the cultivation time, as well as dynamics of the main state variables are presented on the Fig. 5. The substrate concentration again is kept below to the critical concentration with a small period of excess substrate in the broth (cultivation time between 8.75 – 9 h). The cell concentration increases over the cultivation period and its final value is 4.95 gl^{-1} . The obtained biomass concentration is higher compared to the previous results. The GA synthesized feed rate profile using this objective function is superior to above used objective function – sum of the absolute error.

In the case of the objective function – sum of the square time multiplied by absolute error over the cultivation time, it is achieved final biomass concentration of 4.75 gl^{-1} . Although this concentration is lower compared to the above reported results here, the process has reached the desired functional state for a shorter time and maintained this state till end of the process with insignificant increase around 8.75 h of cultivation. The results are presented on Fig. 6.

For all discussed results the calculated feed rate profile leads the process in the desired functional state and maintains the state during the process. In most of the cases there is increase of the substrate concentration above the critical value. These peaks are closely to the critical value, appear for a small periods and could be considered as insignificant.

Considering used objective functions the better results are obtained based on objective functions taking the time into account. Synthesized feed rate profiles are acceptable for the whole cultivation period.

4. CONCLUSION

Cultivation processes are typically very complex, involving different transport phenomena, microbial components and biochemical reactions. Furthermore, the nonlinear behaviour and time-varying properties render difficult process control and optimization. During the fed-batch cultivation the system states change considerably, from a low initial to a very high biomass and product concentration. One of the most important characteristics of cultivation processes, which make more difficult the control design, is the change of cell population state. To reflect these particularities functional state modelling approach is used. For the considered *E. coli* cultivation process as appropriate functional states ensuring an optimal system regime two states are defined. Mixed oxidative state and complete sugar oxidative state are desired functional states.

On the other hand, this dynamic behavior of the cultivation processes motivates the development of optimization methods to find the optimal input feeding trajectories in order to improve the process. Development of a suitable feeding strategy is critical in fed-batch operation. Moreover, synthesis of definite feed rate profile could be ensured entering the process into desired functional state(s) and maintaining it(s) during the process.

Based on genetic algorithms a feed rate profile that hold defined functional state is synthesized. The obtained results have presented the ability of the genetic algorithms to generate an optimal feed rate profile based on defined rules. The genetic algorithm generates feed rate profile that for a short time leads the process to the desired functional state, determined as optimal, and maintains this state during the process. As a result a high final biomass concentration is achieved under optimal conditions for amount and assimilation of the substrate. In the systems there is not accumulation of the residual substrate and any inhibition effects.

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REFERENCES

1. Carrillo-Ureta, G. E., Roberts, P. D. and Becerra, V. M. (2001): Genetic Algorithms for Optimal Control of Beer Fermentation, – In: Proc. of the 2001 IEEE International Symposium on Intelligent Control, Mexico City, Mexico, pp. 391-396.
2. Charbonneau, P. (2002): An Introduction to Genetic Algorithms for Numerical Optimization, Boulder, Colorado, NCAR Technical Note.
3. de Mare L., Cimander, C., Elfving, A. and Hagander, P. (2007): Feeding Strategies for *E. coli* Fermentations Demanding an Enriched Environment, Bioprocess Biosyst Eng, 30, pp. 13-25.
4. Fleming, P. J. and Purshouse, R. C. (2001): Genetic Algorithm in Control Systems Engineering, Department of Automatic Control and Systems Engineering, University of Sheffield, Research Report No. 789.
5. Goldberg, D. (1989): Genetic Algorithms in Search, Optimization and Machine Learning. Addison-Wesley Publishing Company, Reading, Massachusetts.
6. Halme, A., Visala, A. and Kankare, J. (1992): Functional State Concept in Modeling of Biotechnology Processes, Stephanopolus G. and Karim M. N. (Eds.), IFAC Symposium on Modeling and Control of Biotechnology Processes, Pergamon Press, New York, pp. 153-158.
7. Murray-Smith, R. and Johansen, T. A. (1997): Multiple Model Approaches to Modelling and Control, Taylor and Francis.
8. Na, J.-G., Chang, Y. K., Chung, B. H. and Lim, H. C. (2002): Adaptive Optimization of Fed-batch Culture of Yeast by using Genetic Algorithms, Bioprocess and Biosystems Engineering, 24, pp. 299-308.
9. Pencheva, T., Roeva, O. and Hristozov, I. (2006): Functional State Approach to Fermentation Processes Modelling, Tzonkov S., Hitzmann B. (Eds.), Prof. Marin Drinov Academic Publishing House, Sofia.
10. Roeva, O. (2005): Genetic Algorithms for a Parameter Estimation of a Fermentation Process Model: A Comparison, Bioautomation, 3, pp. 19-28.
11. Roeva, O. (2007): Parameter Estimation of a Monod-type Model based on Genetic Algorithms and Sensitivity Analysis, Lecture Notes on Computer Science, Springer, 4818, pp. 601-608.
12. Roeva, O., Kosev, K. and Tzonkov, St. (2007): Functional State Metabolism in *E. coli* Fed-batch Cultivation Processes, Bioautomation, 6, pp. 10-16.

Journal of International Scientific Publication:
Materials, Methods & Technologies, Volume 4, Part 2

13. Roeva, O., Kosev, K. and Tzonkov, St. (2007): Mathematical Description of Functional States in *E. coli* Fed-batch Cultivation Processes, *Bioautomation*, 7, pp. 34-45.
14. Roeva, O., Pencheva, T., Georgieva, Y., Hitzmann, B. and Tzonkov, St. (2004): Implementation of Functional State Approach for Modelling of *Escherichia coli* Fed-batch Cultivation, *Biotechnology and Biotechnological Equipment*, 18(3), pp. 207-214.
15. Ruenglertpanyakul, W., and Bellgardt, K.-H. (1998): Physiological Phase Models for Bioprocesses, In: *Comp. Appl. in Biot.* 7th, Osaka, Japan, May 31 – June 4, pp. 119-122.
16. Samad, N. A. and Ahmad, A. (2006): Comparison Product Optimization Performance of Fed-batch Fermentation Processes for Penicillin G Production, 1st Int. Conf. on Natural Resources Engineering & Technology, 24-25th July, Malaysia, pp. 562-575.
17. Shin, H. S. and Lim, H. C. (2007): Maximization of Metabolite in Fed-batch Cultures Sufficient Conditions for Singular Arc and Optimal Feed Rate Profiles, *Biochemical Eng J.*, 37, pp. 62-74.
18. Smets, I. Y., Claes, J. E., November, E. J., Bastin, G. P. and Van Impe, J. F. (2003): Optimal Adaptive Control of (Bio)chemical Reactors: Past, Present and Future, *Journal of Process Control*, pp. 1-43.
19. Vanichsriratana, W. (2000): Optimization of a Primary Metabolite Fermentation Process: Effect of Cost Factor on the Optimal Feed Rate Control, *Kasetsart J. (Nat. Sci.)*, 34, pp. 495-499.
20. Zhang, X.-Ch., Visala A., Halme A. and Linko, P. (1994): Functional State Modelling Approach for Bioprocesses: Local Models for Aerobic Yeast Growth Processes, *Journal of Process Control*, 4(3), pp. 127-134.