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# Two Approaches to Control of Fed-batch Fermentation Process

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***Abstract:*** *L-lysine is one of the irreplaceable aminoacids whose content in the animal protein is relatively high in comparison to plants, where the content is relatively low. The insufficiently L-lysine quantity in the fodders reduces the biological value of the fodder doses, reduces the weight increase and the further productiveness of the agricultural animals, it increases the fodder quantity, used for a kilogram growth and decreases the product quantity of animal origin. The most effective and cheapest method for the L-lysine biosynthesis (in biological active form) is the microbiological method via a direct fermentation. In this paper it is used an optimization method at the L-lysine production from strain Brevibacterium flavum 22LD – Neuro-dynamic programming. For receiving a feedback and robustness of the optimal control profile the Model predictive control of the L-lysine production is developed.*

***Keywords***: *Model predictive control, L-lysine, Neuro-dynamic programming.*

## Introduction

Amino acids are the basic bioelements of proteins, which are the most important macromolecules for the functions of humans and animals. Out of the 20 *L*-amino acids, which are found worldwide in most of the living organisms, *L-lysine* is one of the 9 amino acids essential for human and animal nutrition. *L-lysine* is useful as medicament, chemical agent, food material (food industry) and feed additive (animal food) [1].

*L-lysine* is an essential amino acid, which means that it is essential to human health but cannot be manufactured by the body. For this reason, *L-lysine* must be obtained from food. Amino acids are the building blocks of protein. *Lysine* is important for proper growth and it plays an essential role in the production of carnitine, a nutrient responsible for converting fatty acids into energy and helping to lower cholesterol. *Lysine* appears to help the body absorb and conserve calcium and it plays an important role in the formation of collagen, a substance important for bones and connective tissues including skin, tendon, and cartilage [2].

If there is too little *lysine* in the diet, kidney stones and other health related problems may develop including fatigue, nausea, dizziness, loss of appetite, agitation, bloodshot eyes, slow growth, anemia, and reproductive disorders. It is extremely rare, however, to obtain insufficient amounts of *lysine* through the diet. Generally, only vegetarians who follow a macrobiotic diet and certain athletes involved in frequent vigorous exercise are at risk for *lysine* deficiency.

*Lysine* is involved in the browning reaction, or carmelization, in foods such as pastries, doughnuts, cookies and cereals. In this process, *lysine* and sugar become linked together in a way that makes *lysine* difficult for the body to absorb. As a result, a diet high in cereals and

baked goods, especially those that contain a lot of simple sugars, can result in low *lysine* intake [8].

*L-lysine* is used in: Herpes and Shingles, Osteoporosis and other - certain forms of *lysine* and/or *lysine* bound to anti-inflammatory medications may help alleviate pain following an episiotomy (a procedure performed during labour that involves cutting the vaginal area to enlarge the vaginal opening and facilitate delivery). These forms of *L-lysine* may also relieve migraine headaches and painful periods [9].

The production of *lysine* is a complex process and one that makes stringent demands regarding sterility of both the plant and the raw ingredients themselves. As *lysine* is produced by bacteriological fermentation, the "*environment*" in which the fermentation takes place must be precisely controlled [9].

*Neuro-dynamic programming* (*NDP*) is suggested as a method for alleviation of the "*curse of dimensionally*" of Dynamic Programming (*DP*). The name *NDP* expresses the reliance of the methods of this article on both *DP* and neural network (*NN*) concepts [2, 3].

*Model predictive control (MPC)* is a general methodology for solving control problems in the time domain. More than 15 years after *MPC* appeared in industry as an effective means to deal with multivariable constrained control problems, a theoretical basis for this technique has started to emerge. The outcomes of feasibility of the *on-line* optimization, stability and performance are largely understood as systems described by linear models. Much progress has been made on these issues for non-linear systems but for practical applications a lot of questions remain, including the reliability and efficiency of the on-line computation scheme. To deal with model uncertainty "*rigorously*" an involved dynamic programming problem must be solved. The approximation techniques proposed for this purpose are largely at a conceptual stage. Among the broader research needs the following areas are identified: multivariable system identification, performance monitoring and diagnostics, non-linear state estimation, and batch system control. Many practical problems, such as control objective prioritization and symptom-aided diagnosis, can be integrated systematically and effectively into the *MPC* framework by expanding the problem formulation to include integer variables yielding a mixed-integer quadratic or linear program [6, 7].

The aim of this study is to develop *Neuro-dynamic optimization* and *Model predictive control* at the *L-lysine* production.

## Materials and methods

### *Process specifics*

The development of a multi-step biotechnological process requires three steps, comprising:

- Identification and characterization of a suitable biological system (microorganism, biocatalyst).
- Increase of bioreactor productivity by systematic media optimization and adaptation of fermentation technology to a developing process (process development and fermentation technology) (Fig. 1).
- Downstream process (cell separation by centrifugation or ultrafiltration, separation, evaporation and drying).

In addition to physical parameters like pH, agitation and aeration rate, air saturation, temperature, dissolved $CO_2$ and foaming, medium composition is a very important factor strongly influencing fermentation processes, often being object of extensive process development and optimization studies. The culture medium must satisfy in a suitable manner the requirements of microbial growth and production.

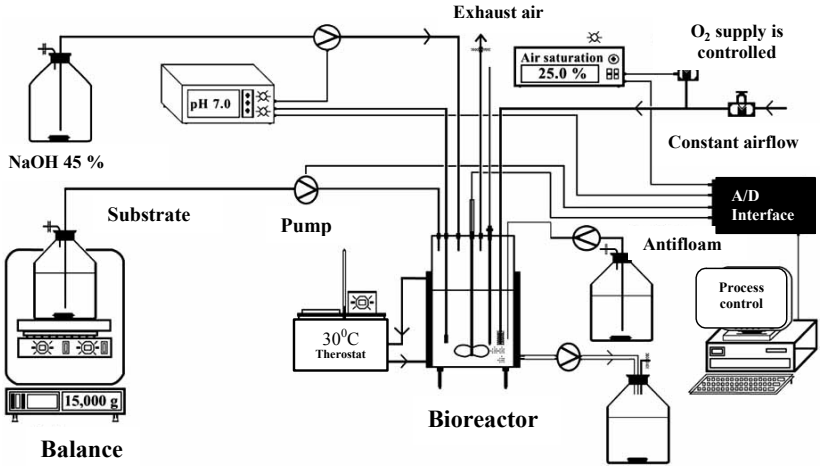


Fig. 1 Bioreactor for the *L-lysine* fermentation

Defined media acquiring pure growth requiring nutrients and essential additives or alternatively undefined media containing natural organic substances such as soybeanhydrolyzate, corn steep liquor, yeast extract or peptone are used for *L-lysine* fermentation. Common fermentation media for *L-lysine* production contain various carbon and nitrogen sources, inorganic ions and trace elements (Fe++, Mn++), amino acids, vitamins (biotin, thiamine-HCl, Nicothin amide) and numerous complex organic compounds. An over expression of genes is also achieved by optimizing the composition of the media and the culture technique in addition to physiological and genetic parameters [5, 11].

Further components are necessarily added to fermentation media at the initiation and/or intermittently during the course of *L-lysine* fermentation, such as inorganic salts of various metals, such as magnesium, calcium, potassium, sodium, iron (e.g. iron sulphate), manganese, and zinc or traces of other metals. Phosphoric acid, potassium dehydrogenate phosphate ($KH_2PO_4$) or dipotassium hydrogen phosphate ($K_2HPO_4$) are commonly used as a source of phosphorus for the production of *L-lysine* [5, 6].

*L-lysine* can be produced either by a chemical or by a biochemical method, which is economic, even though relatively low yields are obtained during the extraction of *L-lysine*, requiring specific installations and the use of expensive products. The stereospecificity of amino acids and the steadily increasing *L-lysine* demand necessitates indispensably their fermentative production (the *L* isomer) over synthetic processes [6].

*Process model*

The process model is:

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

$$\frac{dS}{dt} = \frac{F}{V}(S_{in} - S) - k_5 \mu X - k_6 X - k_7 \eta X$$

$$\frac{dTr}{dt} = \frac{F}{V}(Tr_{in} - Tr) - k_{13} \mu X - \frac{F}{V} Tr \quad (1)$$

$$\frac{dC_L}{dt} = k_l a(C^* - C_L) - k_{14} \mu X - k_{15} X - k_{16} \eta X - \frac{F}{V} C_L$$

$$\frac{dL}{dt} = \eta X \quad and \quad \frac{dV}{dt} = F$$

where: $\mu = \frac{k_1 Tr\ C_L}{[(k_2 + Tr)(k_3 + S_0 - S)(k_4 + C_L)]}$, $h^{-1}$;

$\eta = \frac{k_8 S C_L}{[(k_9 + S)(k_{10} + S)(k_{11} + C_L)(k_{12} + C_L)]}$, $h^{-1}$.

$\mu$ – specific rate of *L-lysine* synthesis, $h^{-1}$; $\eta$ – specific rate of *L-lysine*, $h^{-1}$; $X$ – biomass concentration, $g \cdot l^{-1}$; $L$ – *L-lysine* concentration, $g \cdot l^{-1}$; $S$ – glucose concentration, $g \cdot l^{-1}$; $V$ – working liquid volume, l; $F$ – feed flow rate, $l \cdot h^{-1}$; $Tr$ – *Threonine* concentration, $mg \cdot l^{-1}$; $t$ – process time, h; $C_L$ – dissolved oxygen concentration, $g \cdot l^{-1}$; $C^*$ – equilibrium dissolved oxygen concentration, $g \cdot l^{-1}$; $S_{in}$ – input feed substrate concentration, $g \cdot l^{-1}$; $Tr_{in}$ – input feed *Threonine* concentration, $g \cdot l^{-1}$; $k_l a$ – volumetric liquid mass transfer coefficient, $h^{-1}$.

The initial conditions in the model (1) have the follows values:
$X(0) = X_0 = 3.00\ g \cdot l^{-1}$, $S(0) = S_0 = S_{in} = 100.00\ g \cdot l^{-1}$, $Tr(0) = Tr_0 = Tr_{in} = 100.00\ mg \cdot l^{-1}$,
$C_L(0) = C^* = C_0 = 6.1 \times 10^{-3}\ g \cdot l^{-1}$, $L(0) = 0.00\ g \cdot l^{-1}$, $V(0) = V_0 = 10.00$ l.

The model coefficients in (1) have the following values [12]:
$k_1 = 20.8$ $k_2 = 42.0$ $k_3 = 28.0$ $k_4 = 1.1$ $k_5 = 1.01$ $k_6 = 0.07$ $k_7 = 0.51$
$k_8 = 62.0$ $k_9 = 28.0$ $k_{10} = 37.0$ $k_{11} = 4.0$ $k_{12} = 0.12$ $k_{13} = 6.10$ $k_{14} = 448.0$
$k_{15} = 22.0$ $k_{16} = 209.0$ $k_l a = 120$

## Neuro-dynamic optimal control of the process

The objective of this work is to find optimal feed flow rate ($F(t)$) of a fed-batch process such as the *L-lysine* production that will raise *L-lysine* at the end of the process, i.e.:

$$\max_{\mathbf{u}} Q = \int_{t_0}^{t_f} L(t)V(t)\,dt \quad (2)$$

where: $t_0$ – initial time, $t_f$ – final time of the fermentation.

The control objective is, therefore, to drive the reactor from the low product steady state to the desirable high product rate. It may be considered as a step change in the set point at time $t = 0$ from the low product concentration to the high product concentration steady state.

*NDP* uses neural network approximations for approximation of *cost-to-go* function. The *cost-to-go* function was not used to generate an explicit control law; instead, it was used in *on-line*

optimization to reduce a large (or infinite) horizon problem to a relatively short horizon problem. The method was found to be robust to approximation errors. Both deterministic (step changes in kinetic parameters) and stochastic problems (random variations in kinetic parameters and feed composition) were explored [1, 2].

The simulation-based approach involves computation of the converged *profit-to-go* approximation *off-line*. The following steps describe the general procedure of *NDP* algorithm:

1. Perform simulations of the process with chosen suboptimal policies under all representative operating conditions. Starting with a given policy (some rule for choosing a decision *u* at each possible state *i*), and approximately evaluate the cost of that policy (as a function of the current state) by least-squares-fitting a scoring function $\widetilde{Q}^j(L)$ to the results of many simulated system trajectories using that policy.
2. The solution of *one-stage-ahead cost* plus *cost-to-go* problem, results in improvement of the cost values [2].
3. The deviation, which is a result of the optimality, depends on a variety of factors, principal among which is the ability of the architecture $\widetilde{Q}^j(L)$ to approximate accurately the cost functions of various policies.
4. *Cost-to-go* function is calculated using the simulation data for each state visited during the simulation, as for each closed loop simulation (simulation part).
5. A new policy is then defined by minimization of Bellman's equation, where the optimal cost is replaced by the calculated scoring function, and the process repeats. This type of algorithm typically generates a sequence of policies that eventually oscillate in a neighbourhood of an optimal policy.
6. Fit a neural network function approximator to the data to approximate *cost-to-go* function as a smooth function of the states.
7. The improved costs are again fitted to a neural network, as described above, to obtain subsequent iterations $\widetilde{Q}^1(L)$, $\widetilde{Q}^2(L)$, and so on …, until convergence is accomplished.
8. Policy update may sometimes be necessary to increase the coverage of the state space. In this case, more suboptimal simulations with the updated policy are used to increase the coverage or the number of data points in certain region of state space.

The previous points are used in this algorithm: $Q(t_k)$ represents *cost-to-go* values for a stage *k*, $\widetilde{Q}$ represents functional approximation of *cost-to-go*, superscript *j* represents iteration index.

The next values of *F* are examined:
$F \in [0.1,\ 0.2,\ 0.4,\ 0.5,\ 0.7]$,
that can cover the possible rang of variations.

Improvement to the *cost-to-go* function is obtained through iterations of the Bellman equation. This method is known as value iteration (or value iteration). The solution of the *one-stage-ahead* cost plus *cost-to-go* problem, results in improvement of the cost values. The improved prices were again fitted to a neural network, as described above, to obtain subsequent iterations $\widetilde{Q}^j(L)$, $\widetilde{Q}^2(L)$ and so on ..., until they are converged. Cost is said to be "converged" if the sum of the absolute error is less than 5% of the maximum cost. The cost is converged in 7 iterations for our system.

The converged *cost-to-go* function from above was used in solving the one-stage-ahead problem. The choice for switch over the one-stage-ahead is calculated with:

$$\mathbf{u}(k) = \arg \max_{\mathbf{u}(k)} \left\{ f \left( \frac{Q(t_k)}{t_k}, \mathbf{u} \right) + \tilde{B}^6 \left( \frac{Q(t_k)}{t_k}, \mathbf{u}(k) \right) \right\}, \quad (3)$$

where: **u** is vector of control variables, *k* is the optimization stages, *B* is Bellman equation.

Following this procedure, a program on *MATLAB 7.0* is developed and the optimal profile of the optimal control variable can be obtained using it.

The optimal value of feed flow rate is shown in Fig. 2.

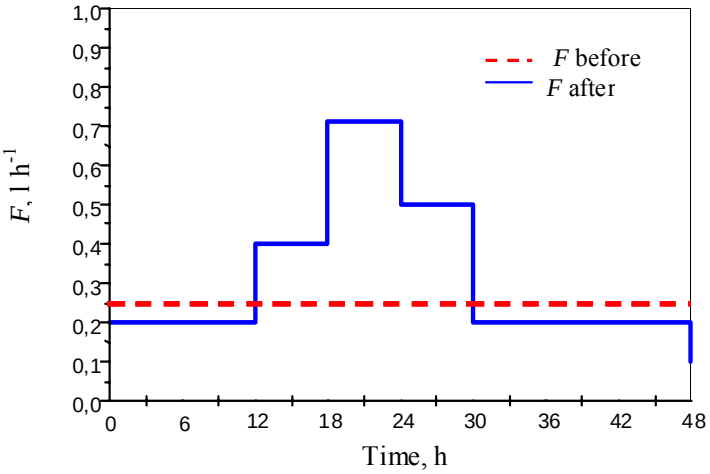


Fig. 2 Optimal feed flow rate

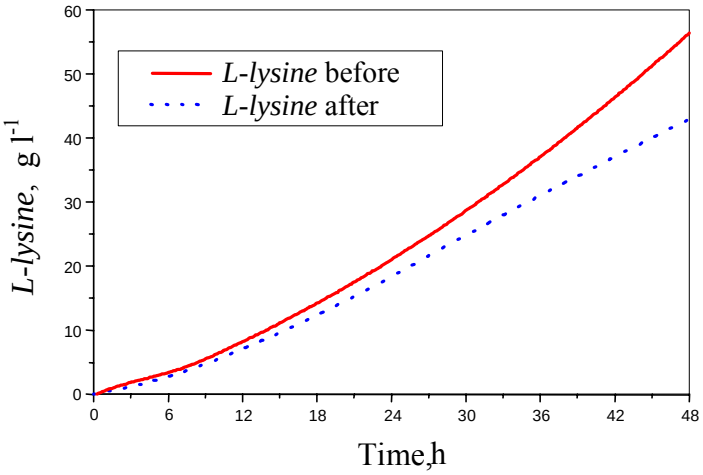


Fig. 3 *L-lysine* concentration before and after optimization

The *L-lysine* production before and after optimization is shown in Fig. 3. Fig. 3 shows increase of the *L-lysine* after optimization by 39.41%.

At this point, an optimal profile of the feeding rate is received (Fig. 2) and with this profile an optimized *L-lysine* production is obtained. However, the optimization algorithm does not have

a feedback and it does not guarantee robustness to process disturbances. Because of that, *MPC* will be developed that will guarantee robustness to process disturbances.

## Model predictive control

Models in *MPC* are used for predicting the process output over a prediction horizon. Control actions are calculated over a control horizon in such a way that the predicted process output is as close as possible to the desired reference signal, and the first control action in sequence is applied in each step (Fig. 4) [7, 10].

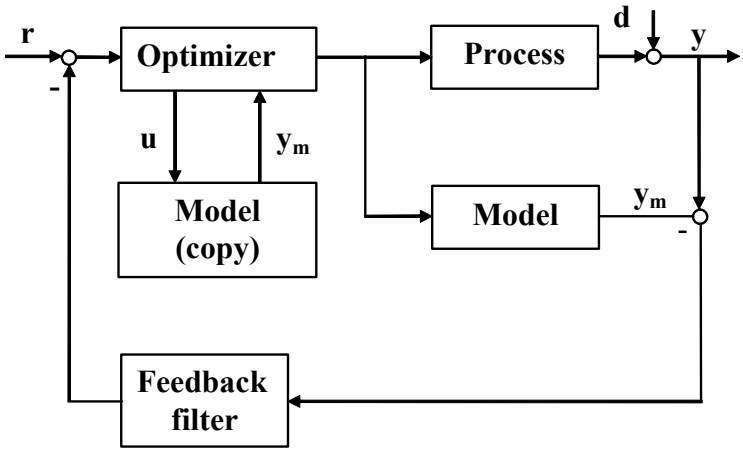


Fig. 4 Internal model predictive control

The control of fermentation processes focuses on an open-loop operation owing to their highly nonlinear and inherently difficult dynamic behavior. Optimization is carried out *off-line*, and the reactor is fed with the determined optimal feed profile. Once the batch proceeds there is no provision to account for disturbances occurring during the batch.

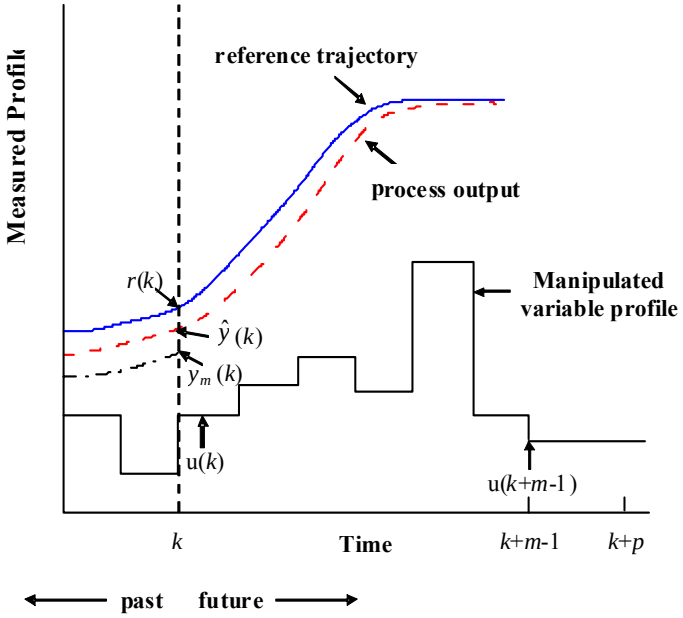


Fig. 5 *MPS* algorithm scheme

This problem of implementing the optimal policy *on-line* and ensuring that the system follows the optimal trajectory in the presence of disturbances has received limited prior consideration.

Attention is thus focused on designing a controller to track the optimal policy, and on approaches for disturbance compensation for the closed-loop control problem [10].

In order to understand *MPC* algorithm, consider Fig. 5. The figure and the notation used in the description are adapted from [7, 10]. The first part of the *MPC* algorithm is the specification of the reference trajectory, which may be as simple as a step change to a new setpoint or, as it is common for batch processes, a trajectory that the system must follow. At the present time, $k$, the reference trajectory has a value $r(k)$.

Also at $k$, consider the predicted process output over a future prediction horizon, $p$. A suitable controller model of the process is used to obtain the projected behavior of the output over the prediction horizon by simulating the effects of past inputs applied to the actual process (value $\hat{y}(k)$ at the current time).

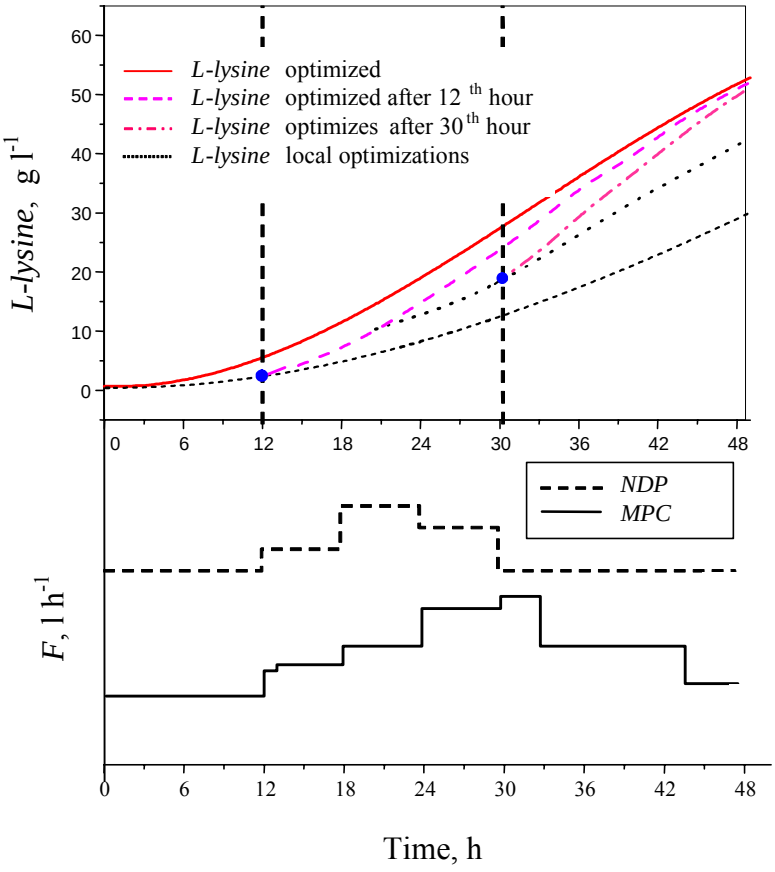


Fig. 6 *MPC* to *L-lysine* production

The same controller model is used to calculate a sequence of $m$ current and future manipulated variable moves, in order to satisfy a specified objective function. Here, $m$ is the move horizon. A common objective function is to minimize the sum of squared deviations of the predicted controlled variable values from a time-varying reference trajectory, over the prediction horizon, based on system information available at the current time $k$. The minimization also takes into account constraints that may be present on the state, output, or the manipulated variable. Conceptually, the problem is similar to constructing and utilizing

the inverse of the controller model to determine the sequence of *m* moves that most closely achieves the specified output behavior over the predicted horizon [10].

However, due to unmodeled disturbances and modelling errors, there may be deviations between the actual observed output, $y_m(k)$ and the predicted output behavior. Due to these deviations, the computed future manipulated variable moves may no longer be appropriate and hence, only the first of the computed manipulated variable moves, $\Delta\mathbf{u}(k)$, is implemented on the actual process. The error $d(k) = y_m(k) - \hat{y}(k)$, is calculated and is used to update the future measurements. At the next time instant, $k + 1$, the process measurement is again taken and the horizon is shifted forward by one step. Based on this new horizon and using the updated system information, the optimization is carried out again, and the process continues. Since, the horizon recedes, at the next time step, it is also known as a receding horizon control problem.

An algorithm for application of *MPC* for the investigated process is developed. The results are shown on Fig. 6. For a first control point is chosen the 12$^{th}$ hour. As it may be remarked there is a diversion from the reference profile, marked on the figure with "*red*", accordingly the optimal profile is changed – *MPC* profile. The second point is at 30$^{th}$ hour. The obtained control guarantees the robustness and stability of the *L-lysine* production.

The local optimizations at the 12$^{th}$ hour and at the 30$^{th}$ hour are made with *NDP* that has been written in Section "Neuro-dynamic optimal control of the process". The optimization criterion is (2) – maximal *L-lysine* concentration at the end of the process.

## Conclusions

An approach for optimal control of fermentation processes, based on *NDP* for a *L-lysine* fed-batch fermentation is developed. It is proposed as methodic for alleviation of "*curse of dimensionally*" of dynamic programming. The results show that quality of *L-lysine* is raised at the end of the process. This optimization method does not have a feedback and it does not guarantee robustness to process disturbances.

*MPC* is developed for guarantee robustness to process disturbances. The method is carried out with an aim control of disturbance of the optimal control variable (feeding rate). For local optimization of each choice optimization hour, *NDP* control algorithm is applied in order to find an optimal profile of the control variable.

The result shows that *NDP* is a convenient and easy to use application method for optimal control that gives the necessary optimal profile, but it does not give robustness of the optimization systems. The developed control algorithm – combined *NDP* and *MPC* ensures maximal quality *L-lysine* concentration at the end of the process and guarantees a feedback on disturbance as well as robustness to process disturbances.

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