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# Intelligent Optimization Method for Batch Fermentation Processes' Initial Conditions

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## Abstract

*The paper considers the problem of initial medium condition's optimization for batch fermentation process - starter culture cultivation in milk industry. The backpropagation through time method is used as optimization procedure for finding the optimal initial values of dissolved oxygen concentration in the milk and concentrations of both strains in the mixed culture with respect to predefined technological demand of final ratio between two microorganisms in the obtained starter culture. The simulation results confirm experimental experience and expert's opinion on the possible optimal initial conditions.*

**Key words:** *process optimization, neural networks, backpropagation, mixed culture, yogurt starter.*

## Introduction

The batch fermentations are a class of biotechnological processes with the following specifics: they don't receive any incoming streams of feeding compounds and don't output products before finishing the cultivation. The only control actions are restricted to the optimal profiles of the physical and chemical conditions in the cultural medium and initial values of the main process state variables. As these process include living microorganisms, their models are highly nonlinear. Moreover the physical and chemical conditions in the cultural medium reflect on their model parameters in complex nonlinear manner that is difficult to describe mathematically sometimes. Hence the optimization of these parameters is usually a subject of experimental experience and accumulated knowledge about the process's specifics.

By now the artificial neural networks' techniques are widely applied to modeling and optimization of biotechnological processes [2]. The initial culture medium parameters optimization problems were usually solved by response surface methodology [4, 5] where a predictive model of combine effect of independent variables is second order polynomial function. It is demonstrated however that this model is outperformed by neural network models in combination with swarm optimization techniques [6] or genetic algorithms [4]. In [7] backpropagation trough time method [15] was successively applied to temperature time profile optimization of a batch biotechnological process. This intelligent optimization approach was further modified and successively applied to determine the initial concentration of dissolved oxygen for the batch fermentation process of yogurt starter culture formation using associated mixed culture [8] where the initial microorganisms' concentrations can't be changed. Here we develop further and apply that approach to optimization of more than one culture initial conditions - microorganisms' initial concentrations together with initial dissolved oxygen concentration - for defined mixed culture in which both microorganisms are cultivated separately and mixed just before inoculation. The process is initially modeled by artificial neural network and then the backpropagation through time method is applied to find the optimal initial culture conditions of the process.

The obtained results demonstrate how the intelligent optimization method applied here can solve complex nonlinear optimization tasks combining black-box modeling approach with the learning abilities of artificial neural networks. The obtained results confirm the experience and knowledge accumulated during experimental work about the process under consideration.

## Experimental set-up

Natural strains *S.thermophilus* 13a and *Lb. bulgaricus* 2-11 are isolated from home-made yogurts manufactured in Rodopite – mountain region in Bulgaria. A new highly effective symbiotic starter culture with high degree of proto-cooperation between strains and high technological characteristics for production of original Bulgarian yogurt is developed from *S.thermophilus* 13a *and* Lb. *bulgaricus* 2-11 [1, 11-14].

The inoculums of monocultures are prepared in the following way: after microbial and biochemical indexes control the whole cow milk is sterilized at 121$^{o}$C for 15 minutes, then the milk is cooled to 43$^{0}$C and is inoculated with 2% of the corresponding culture. Batch cultivations of starter culture *S.thermophilus* 13a + *Lb. bulgaricus* 2-11 are carried out in bioreactor MBR AG Ltd. (Switzerland) with geometric volume 2 dm$^3$ and control device IMCS – 2000.

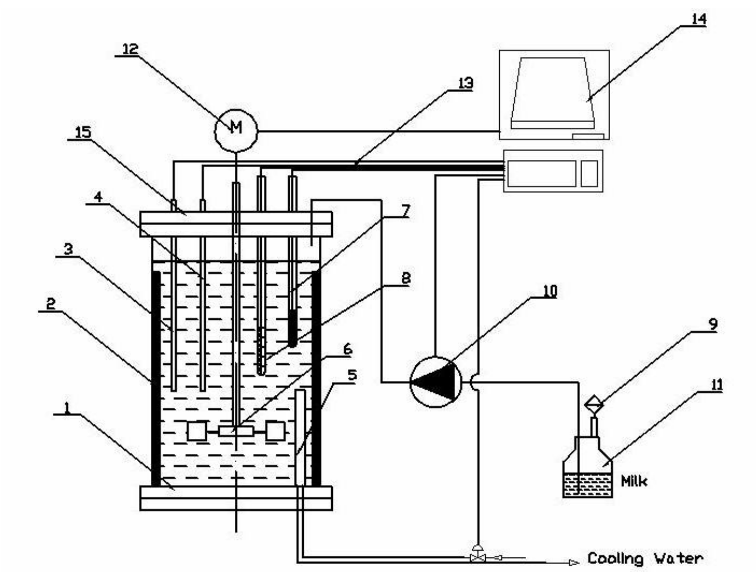


**Fig 1.** Laboratory bioreactor MBR AG Ltd:

1 - apparatus with geometric volume 2 dm$^3$; 2 - four repulse devises; 3 – thermo-strength Pt100; 4 – heater; 5 - heat exchanger for cold water; 6 – stirrer; 7 – pH electrode; 8 – dissolved oxygen electrode; 9 – filter; 10 – peristaltic pump; 11 – flask with sterilized milk; 12 – motor; 13 - control links; 14 – control device.

The laboratory bioreactor MBR AG Ltd. is shown on Figure 1. It is equipped with a six-blade turbine stirrer and four repulse devices. There are two orifices on the lid: one for feeding and the other for the installation of heat exchangers, sensors for temperature, pH and dissolved oxygen. The installation includes sensors and mechanisms for monitoring and control of main physical and chemical process variables – pH, dissolved oxygen concentration and stirrer speed. The feed (sterilized and restored dry milk containing 12% of dry material) and inoculates are inserted in the apparatus using peristaltic pump. Dissolved oxygen concentration could be set using the oxygen control scheme that includes steam-sterilized electrode type „Clark" Ingold. The control system is calibrated in distilled water. The measurements are done in percentage of saturation.

The milk before fermentation is put in a thermostat at the temperature of $43^0$C to be coagulated. The coagulants are kept refrigerated at $4^0$C. During the fermentation, the following biochemical state variables are measured off-line: concentration of lactobacillus plus streptococci (CFU $ml^{-1}$), concentration of substrate lactose (g $l^{-1}$) and the concentration of lactic acid (g $l^{-1}$). Samples are taken at equal time intervals - 0.5 hour. Each experiment is carried out till the total acidity of the medium reaches $80^0$T and then the process is stopped. The number of viable cells of lactic acid bacteria is measured in CFU $(cm^3)^{-1}$ using IDF Standard 117B, 1977. The lactic acid, lactose are measured by enzyme methods (UV test Boehringer Mannheim, GmbH Biochemica).

The final ratio between two species in the starter culture is defined technologically. It is proven that the best ratio is 3:1 streptococcus/lactobacillus. It also depends on the initial cultivation conditions. So the problem of optimal initial concentration of the dissolved oxygen in the milk as well as of initial concentrations of both microorganisms with respect the desirable final ratio between both species is topical problem that has to be decided not only by experiments but also from the control theory point of view.

## Optimization procedure

The backpropagation method was developed initially as procedure for error derivatives calculation in training procedure of artificial neural networks [10]. From a more common point of view it is method for a given function derivative calculations with respect to variables of an ordered system of equations [9, 16]. Hence it could be applied to any optimization problem that can be described in appropriate way.

The optimization task as in [8] is defined as follows: find values of control inputs that minimize/maximize the performance function:

$$J(k) = J(X(k)), \tag{1}$$

where $X$ is vector of object's state variables described by a discrete-time model:

$$X(k+1) = F(X(k), u(k), p). \tag{2}$$

Here, $u$ is vector of control inputs, $p$ – model's parameters vector, $F$ – nonlinear function and $k$ is discrete time variable.

Every optimization procedure needs calculation of performance function's derivatives with respect to the optimized variables. Using the chain rule form [15] they are calculated as follows:

$$\partial X(k) = \frac{\partial^* J(k)}{\partial X(k)} = \frac{\partial J(k)}{\partial X(k)} + \frac{\partial^* J(k+1)}{\partial X(k+1)} * \frac{\partial F(k)}{\partial X(k)}, \tag{3}$$

$$\partial u(k) = \frac{\partial^* J(k)}{\partial u(k)} = \frac{\partial^* J(k+1)}{\partial X(k+1)} * \frac{\partial F(k)}{\partial u(k)}. \tag{4}$$

Here upper star denotes the ordered derivative of $J$ with respect to the corresponding variable. In this way if the optimization task is with defined end, i.e. we know the final time $T$ of the process, the calculation of all ordered derivatives for all times from *0* to *T* is called backwards by backpropagation trough time [7, 15] as follows:

1) For $k=T$ find

$$\partial X(T)=\frac{\partial^* J(T)}{\partial X(T)}=\frac{\partial J(T)}{\partial X(T)}. \tag{5}$$

2) For $k=T-1$ trough zero use equations (3) and (4) to calculate the rest of derivatives.

The optimization procedure scheme is shown on Figure 2. Here the dashed line represents the backwards error derivatives calculations.

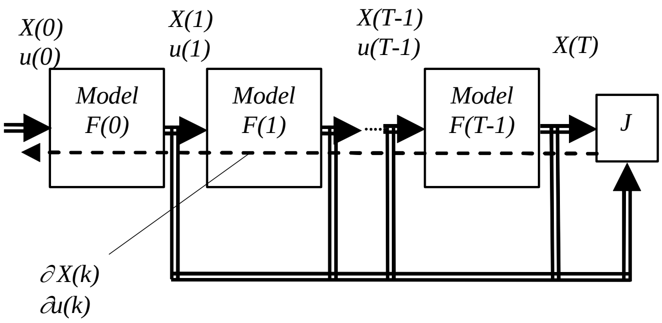


**Fig. 2.** Optimization procedure scheme.

Further every gradient optimization procedure could be applied to fine optimal values of the control variables trough all the time using calculated derivatives. The common iterative optimization procedure is described as follows [3]:

$$\xi_i(k)=\xi_{i-1}(k)\pm\alpha*\Delta\xi_i(k), \tag{6}$$

where $\alpha$ is parameter called learning speed and $\Delta\xi_i(k)$ is step-change of $\xi(k)$ for the $i^{th}$ iteration. Here $\xi$ stands for the optimized variable. In our case $\xi(k)=[ST(k)\quad LB(k)\quad DO(0)]$. The step change $\Delta\xi_i(k)$ is calculated as follows:

$$\Delta\xi_i(k)=g(\partial\xi_i(k)). \tag{7}$$

Here *g* is some dependence of control variable's derivative. Usually it is proportional to the derivative $\partial\xi_i(k)$ but can also depend on the old values of the $\Delta\xi_i(k)$.

The described above optimization procedure is applied to the problem of finding optimal initial value of dissolved oxygen in the milk for the batch starter yogurt culture cultivation process. First we need a process model that will allow us to calculate trough it all the derivatives (3). For that purpose an artificial neural network model of the process is trained. It is layered neural network with one input, one output and one hidden layer of sigmoid output neurons shown on Figure 3.

For the $k^{th}$ time step the input variables vector consists of 4 process's state variables – lactic acid (*LA*), lactose (*L*), *S. thermophilus* 13a (*ST*) and *Lb. bulgaricus* 2-11 (*LB*) concentrations and two variables indicating the chemical conditions in culture – the *pH* of the milk and the initial dissolved oxygen concentration *DO(0)*. The output consists of the process's state vector plus *pH* value for the (k+1)th moment of time. For the neural network model's training several batches of experimental data were used each with different initial dissolved oxygen concentration (15%, 20%, 30%, 40%, 45%, 50%, 60%, 70% and 90%).

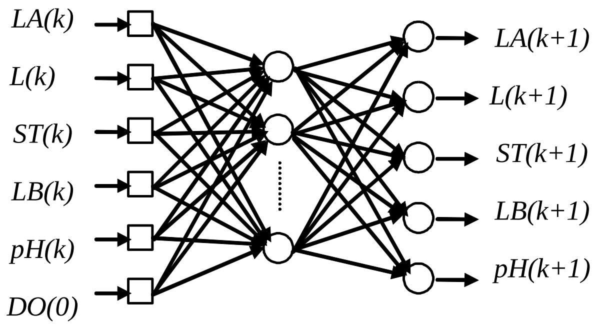


**Fig. 3.** Process's neural network model.

The experimental data are derived into training and testing groups and the trained with training data model is validated using testing once. The obtained model is proved to be correct enough so it can be used further for process simulation during optimization procedure.

The performance criteria used in our investigation was the ratio between two microorganisms in the culture at the end of the process, i.e.:

$$J(T)=\left(set\_po\text{int}-\frac{ST(T)}{LB(T)}\right)^2 . \tag{8}$$

Here the *set_point=3* is predefined technologically desired value. *ST* and *LB* are the concentrations of the two microorganisms (*S.thermophilus* 13a and *Lb. bulgaricus* 2-11 respectively). In order to achieve the desired set point value the performance criteria must be minimized. The initial dissolved oxygen concentration *DO(0)* is considered as constant control variable, i.e. $u=u(k)=DO(0)$. Since here $J$ doesn't depend on the state variables for all time instants but only form the final one and the optimization targets are only initial values of the state variables, for the ith iteration equations (3) and (4) become as follows:

$$\partial X_i=\frac{\partial^* J(0)}{\partial X(0)}=\frac{\partial J(T)}{\partial X(T)}*\frac{\partial F(T-1)}{\partial X(T-1)}*\cdots*\frac{\partial F(0)}{\partial X(0)}, \tag{9}$$

$$\partial u_i=\frac{\partial^* J}{\partial u}=\sum_{k=0}^{T-1}\frac{\partial^* J(k+1)}{\partial X(k+1)}*\frac{\partial F(k)}{\partial u} . \tag{10}$$

Keeping in mind that function $F$ from equation (2) is neural network, its derivatives with respect to all state variables and to the control variable as well are calculated according to the backpropagation algorithm [15] too.

The optimization procedure used here (equations (6) and (7)) was the conjugate gradient method [3]. According to it equation (7) becomes:

$$\Delta\xi_i=\partial\xi_i+\beta*\Delta\xi_{i-1}. \tag{11}$$

The parameters $\alpha$ and $\beta$ form equations (6) and (11) are changed at each optimization procedure's iteration according to the Hestenes-Stiefel formula [3]:

$$\beta_i = \partial\xi_i / \Delta\xi_{i-1} \tag{12}$$

and Silva and Almeida adaptive learning rule [3]:

$$\alpha_i = \begin{cases} a\alpha_{i-1} & \partial\xi_i \partial\xi_{i-1} \geq 0 \\ b\alpha_{i-1} & otherwise \end{cases} \tag{13}$$

where $a=1.1$ and $b=0.9$ are procedure's parameters.

## Results and discussions

Initially the optimization procedure was tried to optimize only initial dissoved oxygen concentration. It started with 15% initial dissolved oxygen concentration and took only 8 iterations to find the optimal value *DO(0)=33.55%*. The obtained using that value final ratio between two microorganisms is 3.9852.

Further we tried to optimize only the initial concentration of both strains using calculated optimal initial dissoved oxygen concentration. In only 2 iterations procedure finds that *ST(0)/LB(0)* must be 1 and $ST(0)=LB(0)=0.6*10^8$ CFU/ml. The final ratio *ST(T)/LB(T)* in that case was 3.65.

Then we tried to optimize all three initial conditions together. The optimization procedure needed 80 iterations. The obtained results are: *DO(0)=21.78%*, $ST(0)=LB(0)=0.6*10^8$ CFU/ml and *ST(T)/LB(T)=2.7427*.

The other achieved result is the process's time optimization since at each run the final moment of the process was decided to be when the pH reaches predefined value of 4.1. At this point starts coagulation of the milk so the process has to be stopped. The process after optimization lasts as follows: *T=2.5* hours for the first case, *T=1.5* hours in the second case and *T=3* hours in the last third case.

The obtained results coincide with the experiments carried out. It can be seen from the experimental data that *ST(T)/LB(T)* ratio was closer to 3 for 20% and 30% initial dissolved oxygen concentrations in the milk – it was about 3.23 and 3.98 respectively when pH reaches about 4.1. The experiments also showed that increasing much over 40% of the initial dissolved oxygen concentration deteriorates the process.

## Conclusions

We applied intelligent method approach to the complex and uneasily solvable problem of initial conditions optimization for batch fermentation processes. The used here backpropagation through time procedure and its modification for the case of initial conditions' optimization problem could be used not only for the yogurt starter culture cultivation process but to the whole class of batch process.

The obtained results are promising and will be extended further to other variables initial values optimization as well as to physical and chemical variables optimal profiles calculation.

## Acknowledgments

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# O metodă inteligentă de optimizare a condiţiilor iniţiale asociate proceselor de fermentare discontinue

## Rezumat

*Acest articol are în vedere problema optimizării condiţiilor iniţiale asociate proceselor de fermentare discontinue, cu aplicaţie la industria de procesare a laptelui. Metoda propagării înapoi (în domeniul temporal) este folosită ca instrument de determinare a concentraţiei optime a oxigenului dizolvat în lapte, respectiv a compoziţiei culturii mixte, în raport de restricţiile şi specificaţiile tehnologice specifice acestui proces. Rezultatele simulării confirmă atât opiniile experţilor, cât şi experienţa practică dobândită până în prezent în acest domeniu.*