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Hybrid Modeling and Optimization of Yogurt Starter Culture Continuous Fermentation

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Abstract: *The present paper presents a hybrid model of yogurt starter mixed culture fermentation. The main nonlinearities within a classical structure of continuous process model are replaced by neural networks. The new hybrid model accounts for the dependence of the two microorganisms' kinetics from the on-line measured characteristics of the culture medium – pH. Then the model was used further for calculation of the optimal time profile of pH. The obtained results are with agreement with the experimental once.*

Keywords: *Neural networks, Hybrid modeling, Yogurt starter culture fermentation.*

Introduction

In starters' mass production the continuous microorganisms' cultivation mode has unarguable advantages in comparison with fed-batch cultivation mode: it overcomes numerous difficulties related to the mixed cultures cultivation, provides high productivity and automated process control and hence cheaper production, and creates conditions for production of standardized starters with homogenous properties and biochemical activity [4, 16].

The continuous yogurt bacteria cultivation as mono or mixed cultures is conducted first by Whittier and Rogers [12]. These authors established important dependence between cultural medium active acidity (pH) and the dilution rate. The system that neutralizes produced lactic acid with fresh milk is firstly described by Wilkowske and Fouts [4] and it is classified as pH-stat [11]. The microorganisms' concentration is controlled by the feeding rate based on the information about the pH change rate. The pH-stat regime is conducted in the case of optically non-homogeneous mediums. In this the pH must not be controlled by means of acid or alkali addition into the apparatus [4, 11]. The first and most exhaustive investigations of continuous mode cultivation for Bulgarian starter milk bacteria are conducted by Girginov (1965). Later similar investigations on the continuous cultivation of *S. Thermophilus* + *Lb. bulgaricus* are carried out by several other authors [5-7].

As biotechnological process include living microorganisms, their models are highly nonlinear. Moreover the physical and chemical conditions in the cultural medium reflect on the model's parameters in complex manner that is difficult to describe mathematically sometimes. Hence there is need to develop new mathematical models accounting for such variables as pH, dissolved oxygen concentration etc.

One possible decision offers so called "grey-box" or hybrid modeling. It combines mechanistic (white-box) component comprising the knowledge of the overall process's dynamics and empirical (black-box) component that substitutes the nonlinear subsystems. A possible candidate for black-box component in such hybrid model is some kind of artificial neural network since neural networks are known as good nonlinear dependences' approximates [3]. By now the artificial neural networks' techniques are widely applied to modeling and optimization of biotechnological processes [2]. There are also many examples of hybrid models that incorporate neural networks for specific kinetic rate's models [8, 9].

The process under consideration here is continuous fermentation for yogurt starter culture formation. It is mixed culture consisting of the strains *S. thermophilus* 13a and *Lb. bulgaricus 2-11* in which the symbiotic co-existence of both microorganisms determines the typicality and strict individuality of yogurt. In a previous work [10] the process was modeled by a system of ordinary differential equations with different structures of specific kinetic rates. However there is no one between tested model's structures that accounts for the on-line measurable variables – *pH* of the medium. Hence the main purpose of present work is to model the kinetics of yogurt starter culture using hybrid model including neural networks that account for on-line measurable variable *pH*. The aim is to use the new model for process control synthesis further.

Yogurt starter culture continuous fermentation process

The natural strains *S. thermophilus 13a* and *Lb. bulgaricu*s 2-11 are isolated from home made original fermented milks from Rodopi Mountains. A highly effective symbiotic starter culture consisted of *S. thermophilus 13a and Lb. bulgaricus* 2-11 was developed. It has high extent of proto-cooperation between these two strains and high technological characteristics needed for original Bulgarian yogurt production [1, 13, 14].The inoculums in both cases (mono and mixed cultures) were obtained as follows: full-cream cow milk with checked microbial and biochemical characteristics was sterilized at 121°C for 15 min, then cooled to 43^0C and inoculated with the desired culture [1, 13, 14].

Continuous pH-stat pre-fermentation cultivation of the starter culture *S. thermophilus 13a + Lb. bulgaricus 2-11* in bioreactor MBR AG Ltd. (Switzerland) with geometrical volume of $2dm^3$ and control device IMCS – 2000 was investigated (Fig. 1) [5, 6, 10]. The apparatus was equipped with six-blade turbine stirrer and four baffles. On the head-plate there are mounted sub-pipes used for cultural medium feeding and standing of heat-exchangers and temperature, pH and dissolved oxygen sensors. The installation also includes measurement and control units for the main process variables – pH, temperature, dissolved oxygen concentration and stirrer rotation speed (Fig. 1). The desired constant pH value of the cultural medium was maintained by neutralization with nutritional medium (cow milk). During the experiment the dissolved oxygen concentration was maintained constant (DO_2 = 3%). The dissolved oxygen concentration was measured by sterilizable electrode type „Clark" Ingold. The system was calibrated with stilled water and the obtained data are in percents form the saturation value. The dissolved oxygen concentration was controlled with ±0.2% accuracy. The dilution rate was determined by measuring the outlet volume of the liquid from the bioreactor. The working volume of the apparatus is 1.5 dm^3. The nutritional medium (sterilized dissolved

powder milk with 12% dry compound) and the inoculums are introduced into the apparatus by peristaltic pump 10. The pre-fermented milks are coagulated in thermostat at 44^0C. The obtained coagulants were kept into refrigerator at 4^0C. The partial pressure of the dissolved carbon dioxide in the milk was measured by means of sterilizable potentiometric CO_2-electrode Ingold and amplifier type 525 Ingold (Switzerland). The system was calibrated using the method proposed by Spinnler [15].

During the fermentation the following variables were measured too: lactobacillus and streptococcus concentrations (CFU ml^{-1}); lactic acid concentration (product, P) ($g \cdot l^{-1}$); dilution rate D, h^{-1}. The samples were taken at the system output and were kept in thermostat at 43^0C for further coagulation.

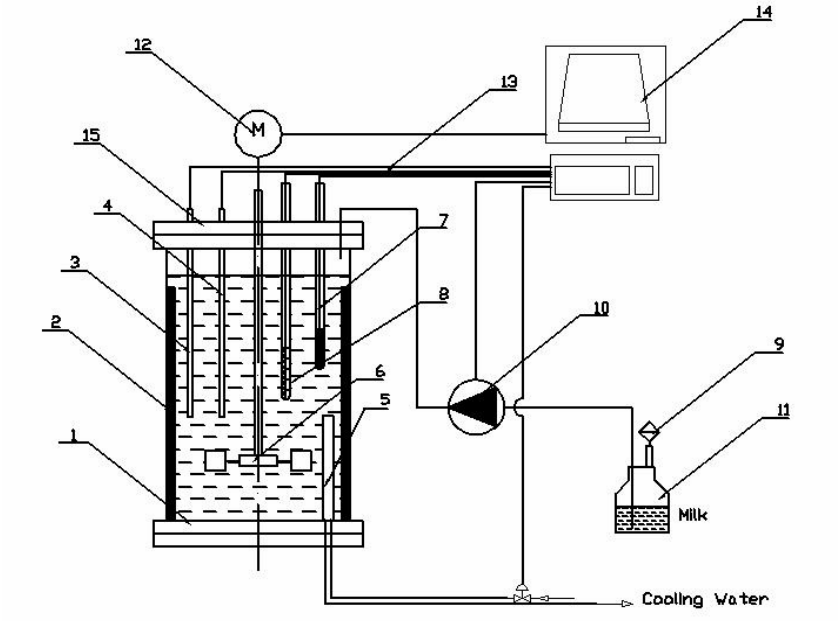


Fig. 1 Laboratory bioreactor MBR AG Ltd

1 – apparatus with geometrical volume 2 dm^3; 2 – baffles; 3 – thermo resistor Pt 100; 4 – heater; 5 – heat-exchanger for cold water; 6 – turbine stirrer; 7 – pH electrode; 8 – oxygen electrode; 9 – filter; 10 – peristaltic pump; 11 – sterilized milk vessel; 12 – drive; 13 – control connections; 14 – control device; 15 – head plate.

Laboratory bioreactor MBR AG Ltd. is shown on Fig. 1. Number of vital lactic acid bacteria cells (CFU, ml^{-1}) is measured by analytical method described in IDF Standard 117B, 1977 and lactic acid (lactose) – by enzyme methods (UV test Boehringer Mannheim, GmbH Biochemica)

Hybrid model of the process

The main structure of a continuous fermentation classical model [7] is the following:

$$\begin{aligned} \frac{dX}{dt} &= \mu(*)X - DX \\ \frac{dP}{dt} &= \varepsilon(*)X - DP \\ \frac{dS}{dt} &= -\eta(*)X + D(S_0 - S) \end{aligned} \tag{1}$$

Here X is biomass (microorganisms') concentration, P is product's concentration, S is limiting substrate concentration and μ, ε and η are nonlinear dependences describing specific kinetic rates of biomass growth, product synthesis and substrate consumption respectively [7], D is dilution rate, S_0 is concentration of substrate in feeding solution (milk). The star in the brackets replaces the process state variables that could influence these specific kinetic rates. There are known numerous structures of μ, ε and η and the choice of proper one is matter of process's specifics.

Here since we have two microorganisms in the culture there must be two separate equations for each biomass concentrations (X_1 and X_2) respectively. Moreover the equations for product (lactic acid) and substrate (lactose) have to be ac accounted for in each microorganism's production and consumption rates of as follows:

$$\begin{aligned}
\frac{dX_1}{dt} &= \mu_1(*)X_1 - DX_1 \\
\frac{dX_2}{dt} &= \mu_2(*)X_2 - DX_2 \\
\frac{dP}{dt} &= \varepsilon_1(*)X_1 + \varepsilon_2(*)X_2 - DP \\
\frac{dS}{dt} &= -\eta_1(*)X_1 - \eta_2(*)X_2 + D(S_0 - S)
\end{aligned} \tag{2}$$

Previously in [6] it was determined that specific production and consumption rates ε and η depend on specific growth rate μ, as follows:

$$\begin{aligned}
\varepsilon(*) &= \frac{\mu(*)}{Y} + m \\
\eta(*) &= a + b\mu(*)
\end{aligned} \tag{3}$$

Here Y, a, b and m are process parameters. Hence there is need to train neural networks only for μ and to use previous knowledge from [14] to compose overall hybrid model.

Since the main on-line measurable variable is pH the purpose of the present work was to account for their influence in the model. Hence the specific growth rate function becomes:

$$\mu(t) = \mu(t, pH(t)) \tag{4}$$

Hence the inputs to both neural networks for specific growth rates of the two microorganisms have to be pH for each time step.

As there are off-line measured data only for the main process state variables (biomass, substrate and product) the specific growth rates have to be calculated from that data. Using the first two equations from the system (2) it is obtained:

$$\mu_1(t) = \frac{1}{X_1(t)} \cdot \frac{\partial X_1}{\partial t} + D$$
$$\mu_2(t) = \frac{1}{X_2(t)} \cdot \frac{\partial X_2}{\partial t} + D \quad (5)$$

Since the experimental data are collected at large time intervals (1 hour) the spline interpolation was used to generate training data for smaller time step (0.1 hours) in order to achieve better results.

The structures of both neural networks for the specific growth rate modeling are feedforward with one hidden layer with 9 neurons. The hidden neurons have log sigmoid transfer function and the one output neuron has hyperbolic tangent sigmoid transfer function. The training procedure was Levenberg-Marquardt backpropagation method.

There are available experimental data for several dilution rates: D = 1.83 h^{-1}, D = 1.86 h^{-1}, D = 2 h^{-1} and D = 2.06 h^{-1}. They are derived into training and testing data sets. The training and testing mean square errors for both trained neural networks are given in Table 1 below.

Table 1. Training and testing errors

	Training error *D=1.83*	**Testing error *D=1.83***	**Training error *D=1.86***	**Testing error *D=1.86***	**Training error *D=2***	**Testing error *D=2***	**Training error *D=2.06***	**Testing error *D=2.06***
1NN	0.0192	0.0258	0.0022	0.1813	0.0007944	0.0130	0.0129	0.0323
2NN	0.0251	0.0359	0.0062	0.1847	0.0088	0.0545	0.0144	0.0878

The trained neural networks for the specific growth rates of both microorganisms in the culture are incorporated in the system equations (2) and the hybrid model is tested with experimental data for all experimental dilution rates. The values of parameters a, b, m and Y are taken from previous work [6]. The mean square errors for all four state variables (two microorganisms', product and substrate concentrations) are given in Table 2 below.

Table 2. Hybrid model errors

D	***D*=1.83 h^{-1}**	***D*=1.86 h^{-1}**	***D*=2 h^{-1}**	***D*=2.06 h^{-1}**
X_1	1.8414×10^{-5}	1.8911×10^{-5}	1.8902×10^{-5}	1.4051×10^{-5}
X_2	4.1875×10^{-5}	4.1477×10^{-5}	3.9424×10^{-5}	3.4326×10^{-5}
S	1.2608×10^{-5}	1.0966×10^{-5}	1.0927×10^{-5}	9.8235×10^{-5}
P	2.1778×10^{-4}	1.6773×10^{-4}	1.8257×10^{-4}	1.6384×10^{-4}

The simulation results for different dilution rates are shown on Figs 12, 3, 4 and 5, respectively. In all figures stars represent the experimental data while the dots – calculated by the hybrid model values. As can be seen from the figures in all cases the new hybrid model approximates very well the process.

The contrast to the model from [10] is that there are no different model parameters' values for different dilution rates. Moreover the hybrid model accounts for the influence of *pH* on the process state variables.

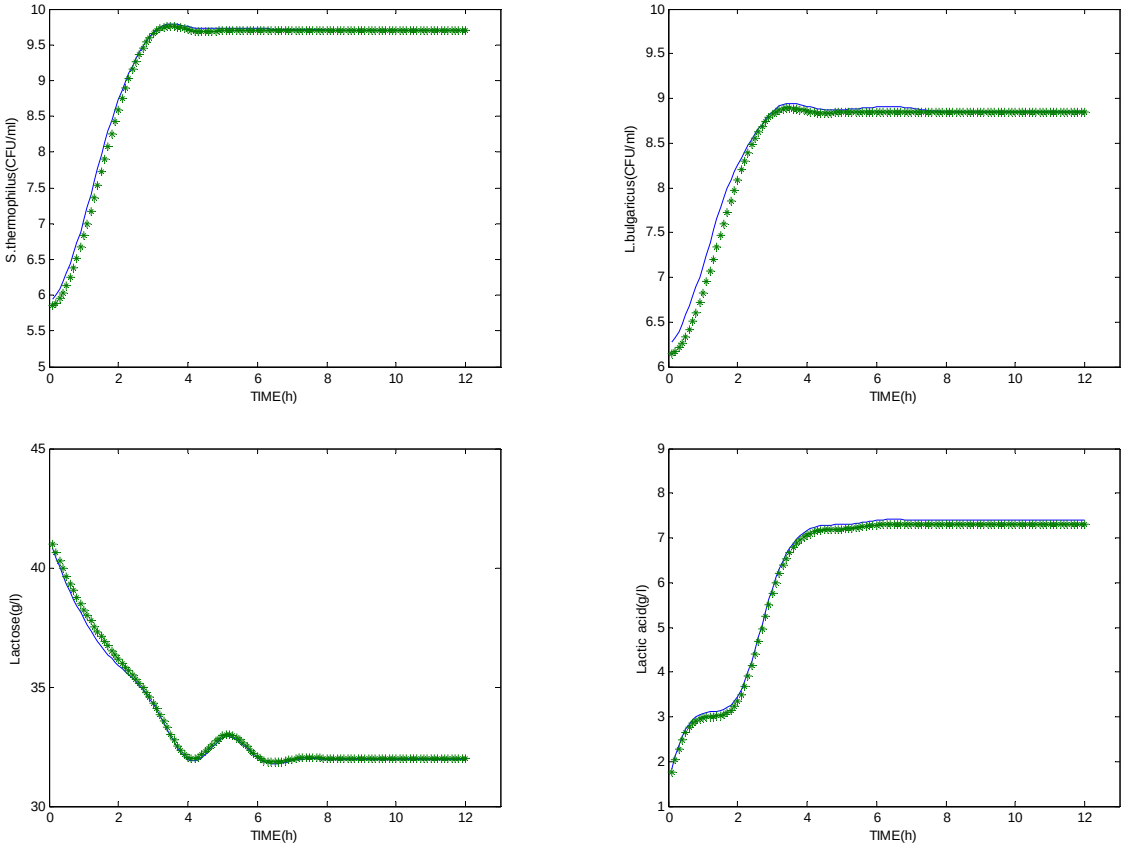


Fig. 2 Simulation results for D = 1.83 h^{-1}

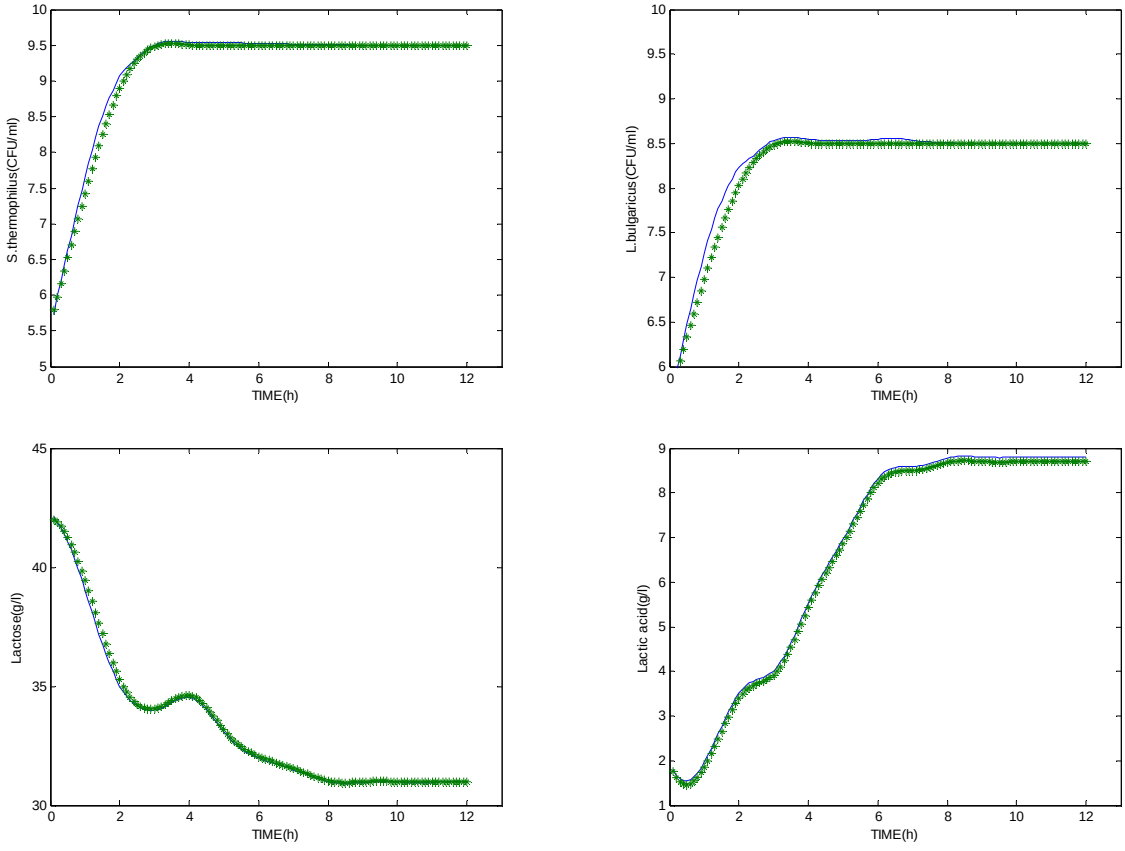


Fig. 3 Simulation results for D = 1.86 h^{-1}

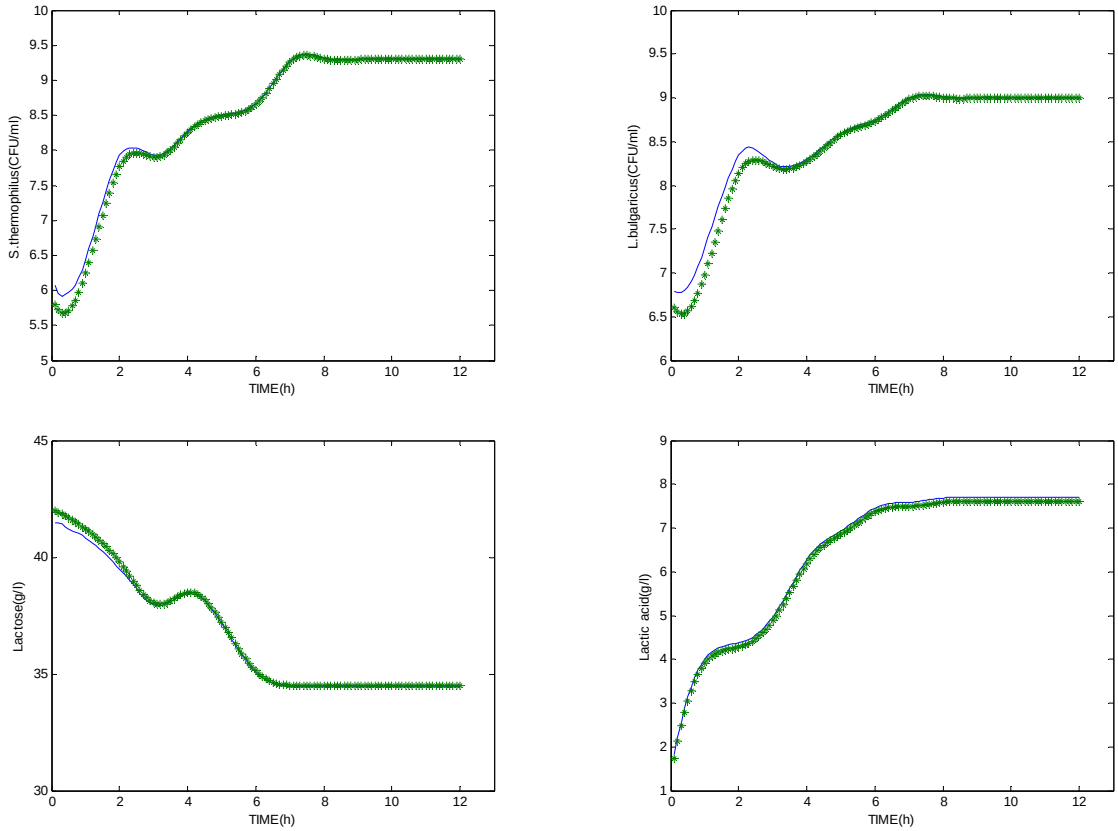


Fig. 4 Simulation results for $D = 2\ h^{-1}$

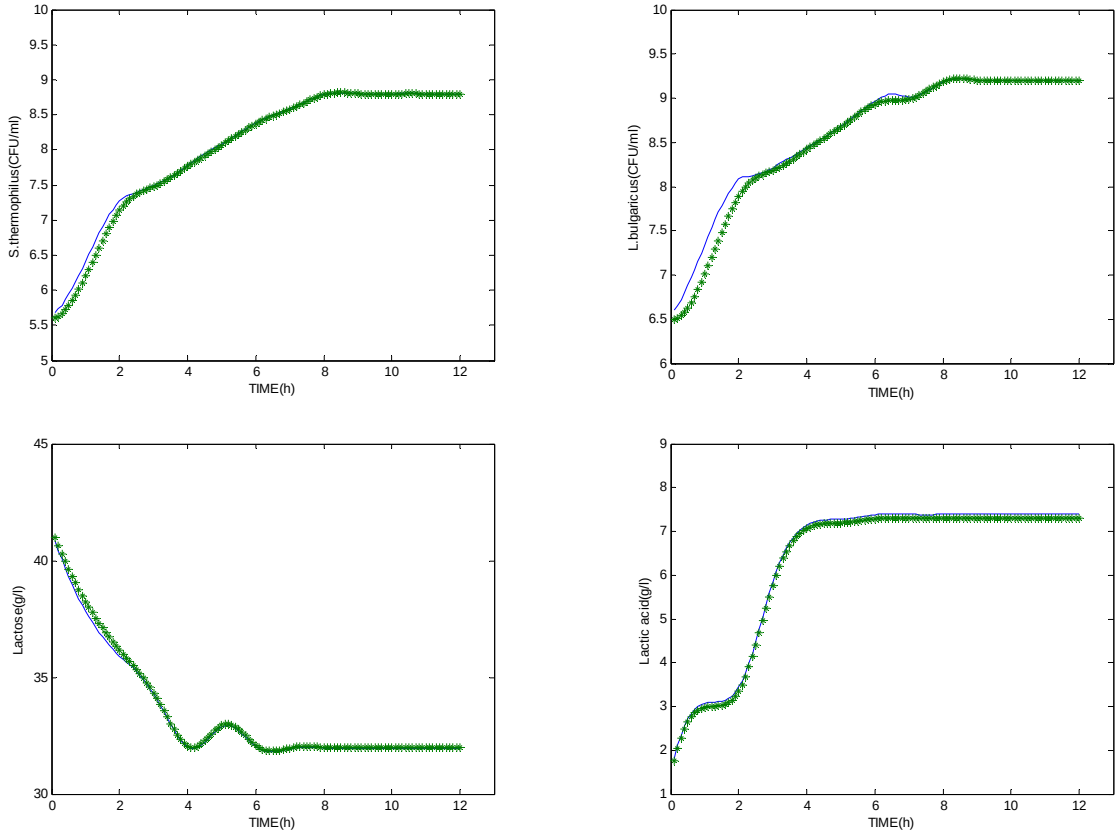


Fig. 5 Simulation results for $D = 2.06\ h^{-1}$

Optimal time profile of *pH*

Since the yogurt quality depends on the final ratio between two microorganisms in the starter it can be a subject of optimization and control. As the only control variable in our case is *pH* that influences directly specific growth rates of both microorganisms the aim is to obtain its optimal value that will yield the desired ratio.

Since the ratio X_1/X_2 has to approach with time the desired value X^*, linearizing feedback control low can be applied as follows:

$$\frac{\partial X_1/X_2}{\partial t} = \lambda\left(X^* - \frac{X_1}{X_2}\right) \tag{6}$$

Here λ is parameter determining the transient time of the closed loop system's dynamics. The following dependence about the ratio between two microorganisms can be derived:

$$\frac{\partial X_1/X_2}{\partial t} = \frac{1}{X_2^2}\left(X_2\frac{\partial X_1}{\partial t} - X_1\frac{\partial X_1}{\partial t}\right) \tag{7}$$

Then by replacing the partial derivatives with first two equations from model (2) and using above two equations it is obtained:

$$\mu_1(pH) - \mu_2(pH) = f(pH) = \lambda\left(\frac{X_2}{X_1}X^* - 1\right) \tag{8}$$

In this way applying the inverse modeling of the time dependence of *f(pH)* from the difference of the two specific growth rates its time trend can be obtained as follows:

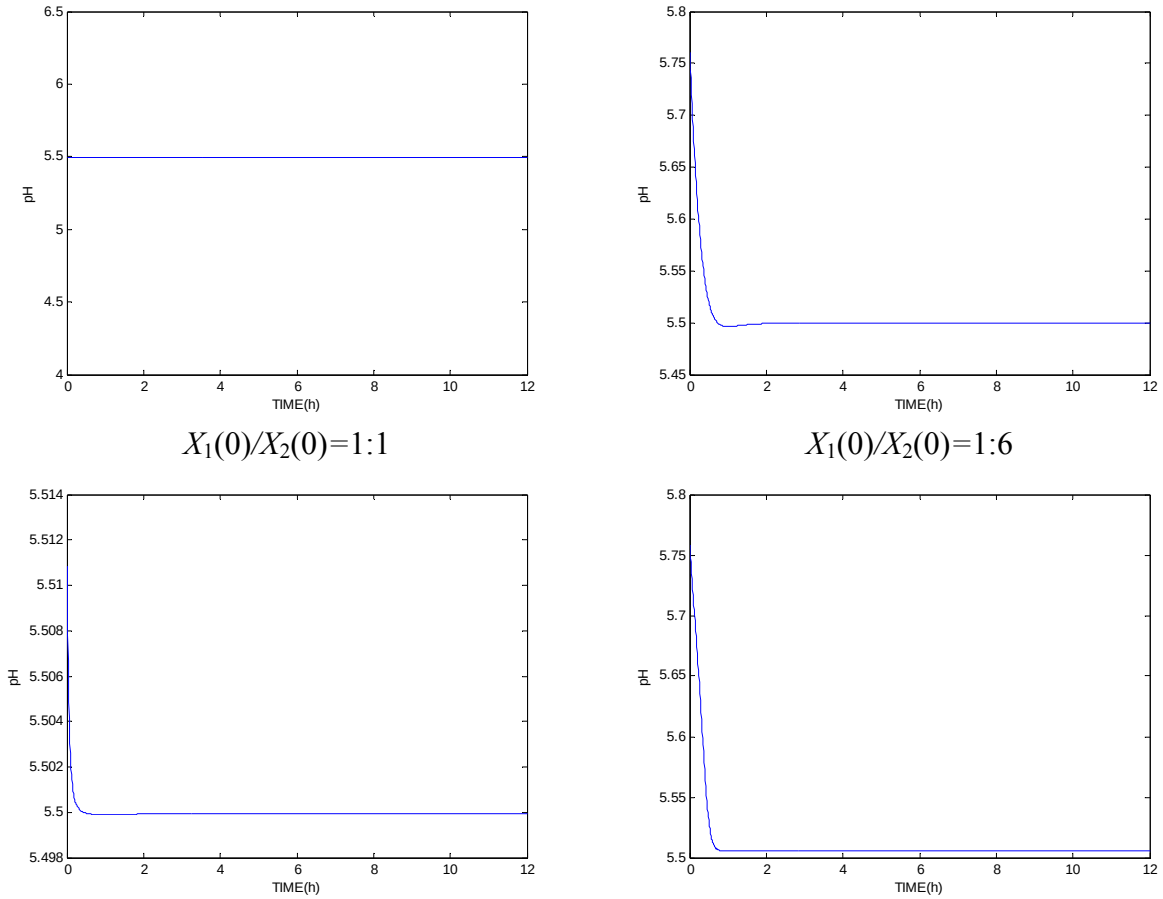
$$pH = f^{-1}(\mu_1(pH) - \mu_2(pH)) = NN(\mu_1 - \mu_2) \tag{9}$$

Here *NN* denotes neural network structure used for inverse modeling. Training of the inverse model is done using the above equation. The neural network structure is with one input, one output and two hidden layers with neuron's numbers 1:7:3:1 respectively. The two hidden layers have logsig transfer functions while the output one – purelin. For the training Levenberg-Marquardt backpropagation method was used.

After inverse *NN* training Eq. (6) was used to calculate desired time trend of the ratio X_1/X_2 for different initial conditions. Then by feeding the dependence:

$$\lambda\left(\frac{X_2}{X_1}X^* - 1\right)$$

to the inverse *NN* model (9) the optimal time trend of *pH* for different initial ratios between two microorganisms was calculated. The obtained results (for $\lambda = 0.2$ and $X^* = 3$) are shown on Fig. 6 below.



$X_1(0)/X_2(0)$=1:1 $X_1(0)/X_2(0)$=1:6

$X_1(0)/X_2(0)$=1:2 $X_1(0)/X_2(0)$=1:8

Fig. 6 Optimal time profiles of *pH*

The obtained results confirm the experimental once. In all cases the optimal value of *pH* for the steady state stage of the process is about 5.5. This value was also determined by experienced technologists who are familiar with the process' peculiarities.

The only difference in the obtained *pH* profiles is in the time when its steady state value has to be reached. Since *pH* influences in different manner the growth rates of the two microorganisms its initial trend (for the transition part of the process) will depend on their initial ratio. As can be seen from the above figures the smaller is value of $X_1(0)/X_2(0)$ the faster must be reached *pH* steady state. This is mainly due to the fact that the set point X^* for the final microorganisms' ratio is closer to the smaller initial ratio between them. The bigger values of *pH* at the beginning of the process will balance both microorganisms' growth rates in such manner that the first microorganism will grow faster and will reach the second one's concentration thus approaching the desired final microorganisms' ratio in the starter.

Conclusions

The proposed new hybrid model of yogurt starter continuous fermentation accounts for the only on-line measurable variable (*pH*) on the specific kinetic rates of the process. Its main advantage is in using neural networks for approximation of nonlinear parts of the model in a black-box manner so there is no need to know or to reveal the complex mathematical relationships of that dependences. The obtained model fits well experimental data for different dilution rates in contrast to the classical mass-balance equations model that has different parameters' values for different dilution rates (as in [10]).

The proposed approach to *pH* time profile optimization allows fast and easy calculation using inverse neural network model of difference between two microorganisms' specific growth rates in dependence on *pH*. The optimization results are with good agreement with experimental once. They can be explained with process's specifics and confirm the expert's opinion as well.

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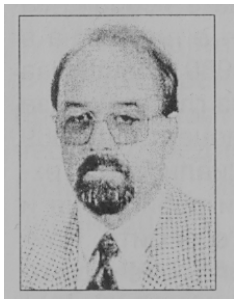

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