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ORIGINAL PAPER

Novel optimal temperature profile for acidification process of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in yoghurt fermentation using artificial neural network and genetic algorithm

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Abstract The acidification behavior of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* for yoghurt production was investigated along temperature profiles within the optimal window of 38–44 °C. For the optimal acidification temperature profile search, an optimization engine module built on a modular artificial neural network (ANN) and genetic algorithm (GA) was used. Fourteen batches of yoghurt fermentations were evaluated using different temperature profiles in order to train and validate the ANN sub-module. The ANN captured the nonlinear relationship between temperature profiles and acidification patterns on training data after 150 epochs. This served as an evaluation function for the GA. The acidification slope of the temperature profile was the performance index. The GA sub-module iteratively evolved better temperature profiles across generations using GA operations. The stopping criterion was met after 11 generations. The optimal profile showed an acidification slope of 0.06117 compared to an initial value of 0.0127 and at a set point sequence of 43, 38, 44, 43, and 39 °C. Laboratory evaluation of three replicates of the GA suggested optimum profile of 43, 38, 44, 43, and 39 °C gave an average slope of 0.04132. The optimization engine used (to be published elsewhere) could effectively search for optimal profiles of different physico-chemical parameters of fermentation processes.

Keywords Process optimization · Temperature profile · Yoghurt acidification profile · Artificial neural network · Genetic algorithm

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Introduction

Yoghurt production is highly interesting in the emerging competitive food sector [3]. In the industrial production process, a starter culture which is a blend of *Streptococcus salivaricus subsp. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* is used in a 1:1 ratio. During the acidification step of the process, *S. thermophilus* grows faster and produces both acid and CO_2 which stimulate the growth of *L. bulgaricus*. Both organisms produce flavoring agents and organic acids which lower the pH to a commercially acceptable value of 4.6 [9]. Coagulation of the milk casein occurs as a result of various organic acids produced during the acidification process. It is well established that temperature among other variables such as the total solid content of the milk, the viability of the starter culture and the inoculum size strongly affects the acidification rate and consequently the product quality [9].

The time-varying nature of microbial fermentation processes has been raised by several authors in different works [2, 4]. For an optimal production, the control set points do not remain constant but follow time-dependent profiles, some kinds of ‘‘randomly moving targets’’[6]. This state raises additional complexities in bioprocess optimization.

Hitherto, the industrial fermentation process of yogurt is carried out at a constant temperature set point of 42 °C for

4–6 h, aeration of 0 vvm, and agitation of 0 rpm. The temperature of 42 °C is a compromise between two optima temperatures of 39 and 44 °C for *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, respectively [14]. Reports on process acidification behavior along a time-varying temperature within the optimal window are scanty. Laligant et al. [10] examined the trends of various variables at 30 and 42 °C during the acidification process of yoghurt using *S. thermophilus* and *Lactobacillus delbrueckii* sub *bulgaricus*.

Traditionally, optimizations of bioprocesses are based on mathematical models, described by a set of differential equations derived from mass balances [12]. These models lack robustness and accuracy due to the physiological complexity of microorganisms [11]. Artificial intelligence tools such as genetic algorithm (GA) and artificial neural network (ANN) provide a new approach to this class of problems. Complex optimization problems can be solved without knowing the impacts of each parameter in detail [8]. The ANNs have the ability to model processes by learning from input/output data, without mathematical knowledge of the process. GAs search for optimal solution to a problem by mimicking the biological evolution of the best-fitted species using genetic operations such as mutations, crossovers and selections. With GA, an optimization problem is treated through a cycle of four stages: creation of population of solutions, selection of the best solutions, evaluation of the solutions, selection of the best solutions and breeding using the parent population and genetic manipulation to create a new population of solutions. The cycle continues until a suitable result is achieved [1]. Duta et al. [5] used the radial basis function (RBF) ANN to build a predictive model for extra protease production from a newly isolated *Pseudomonas* sp. Zuo and Wu [15] proposed a semi real-time strategy for the optimization and control of fed-batch fermentations systems using hybrid neural network and GA.

In this work, a temperature profile which can drive the yoghurt acidification process along an optimal profile is investigated using an ANN model which serves as the evaluation module for the GA search.

Materials and methods

The optimal temperature search was carried out using the optimization search engine module, which is an additional unit of the Biopro-Optimizer software for monitoring and control of microbial fermentation processes [6]. The optimization engine uses an ANN component, which was trained on experimental data for modeling of the process, and a GA sub-unit which iteratively generates better profiles. Using the GA operators and the ANN model for evaluation, optimal profiles ultimately emerge. The details of the engine will be presented elsewhere.

Initialization of the search domain

To generate the initial search domain, the temperature range was set at 38–44 °C, at a changing unit of 1 °C, the process duration time of 5 h, at an interval of 1 h (Fig. 1). With these parameters, the module generated a set of initial time-varying temperature profiles.

Yogurt fermentation process

In order to obtain the training and validating data for the ANN, 14 temperature profiles were randomly selected

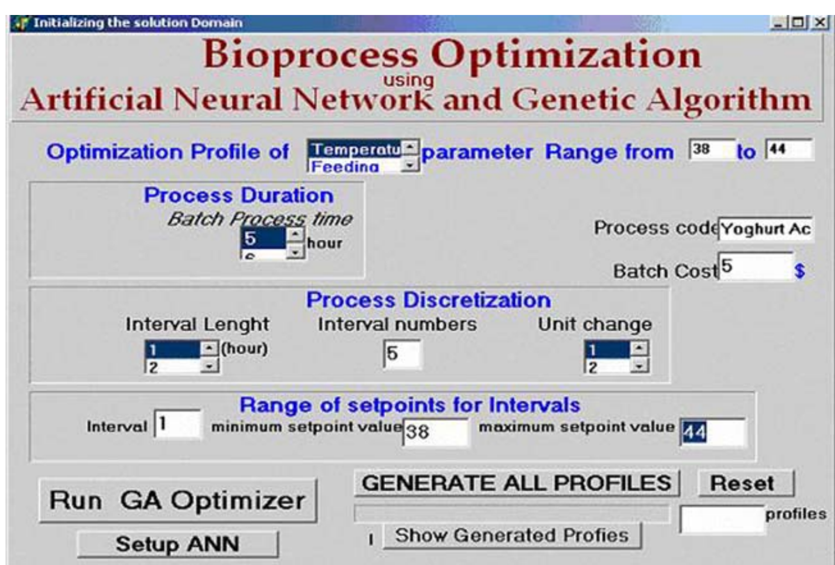


Fig. 1 Initialization panel for the optimization process of yoghurt fermentation

Table 1 Temperature profiles evaluated

Process time (min)	T1	T3	T5	T6	T8	T9	T10	T11	T13	T14	Control
20	43	42	39	45	45	39	45	43	45	45	42
40	43	42	39	45	45	39	45	43	45	45	42
60	43	42	39	45	45	39	45	43	45	45	42
80	38	42	39	38	42	39	41	41	43	43	42
100	38	42	39	38	42	39	41	41	43	43	42
120	38	42	39	38	42	39	41	41	43	43	42
140	44	43	39	39	45	42	41	41	38	38	42
160	44	43	39	39	45	42	41	41	38	38	42
180	44	43	39	39	45	42	41	41	38	38	42
200	43	41	39	44	40	42	38	39	41	41	42
220	43	41	39	44	40	42	38	39	41	41	42
240	43	41	39	44	40	42	38	39	41	41	42
260	42	39	40	45	42	41	45	45	41	45	42
280	42	39	40	45	42	41	45	45	41	45	42
300	42	39	40	45	42	41	45	45	41	45	42

T1–T14 temperature profiles, *control* constant temperature of 42 °C

(Table 1) and evaluated in yogurt fermentation processes. For the starter culture, dried pellets of *L. bulgaricus* and *S. thermophilus* (YC-380, YO Flex, CHR HANSEN A/S Denmark) were used. About 5 g of the starter culture was used to inoculate 250 ml of sterile 40% (w/v) milk (Cowbell, Switzerland) having an average composition of lactose 35%, vegetative fat 28%, proteins 26%, minerals 5.5%, moisture content 3% and sucrose 2%. The mixed culture was incubated at 42 °C for 24 h, after which it was stored at 4 °C and subsequently used as the starter culture for all the batches.

To evaluate the acidification trend of each temperature profile, 40 g of powdered milk was dissolved in 1 l of distilled water. The mixture was heated at 80 °C for 15 min, and then cooled to 38 °C. Two flasks containing 250 ml of the milk slurry were inoculated with 1 ml of the starter culture each, and then incubated in the water bath (Grant Instruments, England, UK). The incubation was done at temperatures predicted by the temperature profile under experimentation. In the control profile, a constant temperature of 42 °C was maintained. Each batch was carried out in duplicates. To monitor the acidification trend, the pH was recorded every 20 min using a pH meter (HI 8519, HANNA Instruments, Singapore) for 5 h. The slope of acidification curve was obtained for each profile, being a function of the performance of the profile. The slope was obtained using Microsoft Excel Package (Microsoft Inc. USA). The package first determines the regression line of the acidification trajectory, and then computes the slope as a vertical distance on pH change divided by a horizontal distance on process time. This is the rate of change along the regression of the acidification trajectory.

Structure of the ANN

A two-layer modular ANN was structured. The first and second layers used sigmoid transfer functions, whereas the output layer used a linear function. The input vector comprises five set point values along a temperature profile, whereas the output is the slope of the acidification curve. To train the ANN, input and output data from 7 out of the 12 experimental batches were exposed to the network. A set of untrained and trained data was used for validation.

GA optimization search

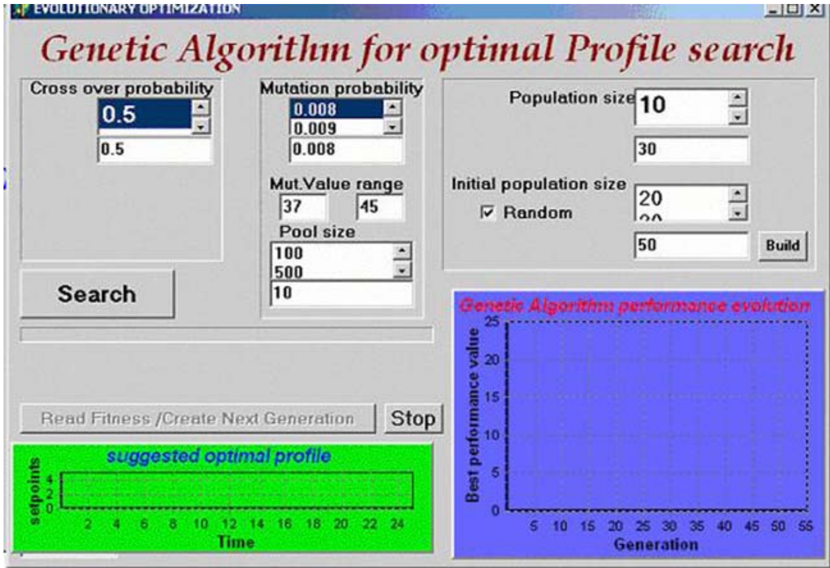
In the GA search panel of the optimization engine, the crossover probability was set at 0.5, the mutation rate at 0.008 and population size was maintained at 30 (Fig 2). GA sub-module randomly selected a set of temperature profiles from the initial set to form the generation 0 (G0). Subsequent generations evolved iteratively after application of GA operations such as mutations, crossover of the profiles, ranking using the performance value, and selection. This was carried out iteratively until an optimal profile emerged or the stopping criterion met.

Results and discussion

Initialization of optimization search domain

The temperature range set in the window of 38–44 °C was chosen considering the previous reports on optimal growth temperatures of 39 and 44 °C for *S. thermophilus* and *L.*

Fig. 2 Setup panel of the genetic optimization search engine



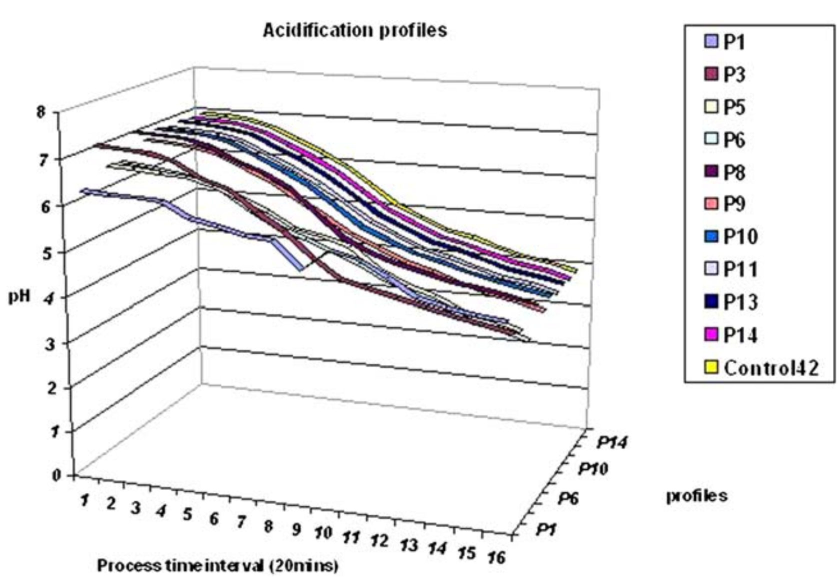
bulgaricus, respectively. With other parameters set as in Fig 1, a total of 16,807 potential optimal profiles were generated. An exhaustive experimental evaluation of these is practically cumbersome, more so if the changing unit of parameter within each interval is set below 1 °C, it will lead to an exponential expansion of the search domain.

Yoghurt fermentation process

Out of the 14 profiles (Table 1) that were randomly selected from the initial set, profiles T2, T4, T7 and T12 were discarded because of the high noise value in their acidification profile, and the remaining 10 profiles and the control were further considered. With regard to the acidification profile of the evaluated temperature profiles, the profile P13 shows the shortest acidification time to reach a pH of 4.5 (Fig 3). However, P3 emerged with the highest slope (0.0127) in the acidification trend (Fig 4). The temperature profile of the emerged P3 shows a sequence of 42, 42, 43, 41 and 39 °C. This may indicate that for a fast acidification, *L. bulgaricus* requires slightly lower temperatures in the terminal phase of the acidification process, whereas *S. thermophilus* initiates the process at higher temperatures.

The temperature profile T1 with a set point sequence of 43, 38, 44, 43 and 42 °C showed the lowest slope (0.00698). It seems that although *S. thermophilus* initialized the acidification process at high temperature, the sudden transition from 38 to 44 °C from the third time interval prevented an optimal acidification. Examination of the temperature profiles T5, T6, T8, T9, T11 and T14 with

Fig. 3 Acidification profiles of investigated temperature profiles



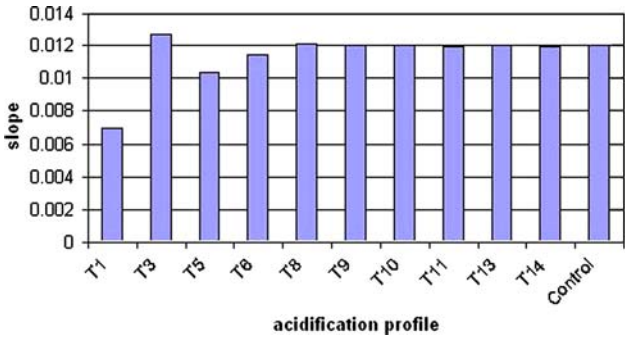


Fig. 4 Slope of the various acidification curves

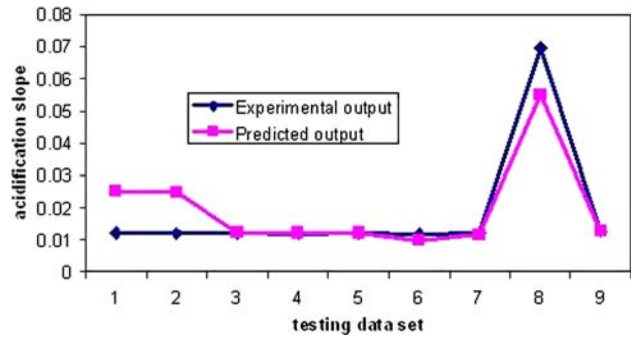


Fig. 5 Predicted acidification slopes versus experimental acidification slopes

acidification slopes of 0.0104, 0.0115, 0.0122, 0.0120, 0.0119, 0.0120 and 0.0119, respectively, against the control experiment (constant temp. = 42 °C) with a slope of 0.0120 (Fig 4) suggests that temperature profiles initiating at a temperature value below 40 °C or terminating at a temperature value above 40 °C do not result in a fast acidification.

Structure and training of the neural network

The modular feed forward neural network used in this work is a special class of multi-layer perceptrons (MLP) which process its inputs using several parallels MLPs. Silva et al. [13] used this structure to model Cephalosporin C production. The topology fosters specialization of function in each sub-module. The absence of full connectivity reduces number of weights thereby speeding up the training process with a small size of training datasets. Seven of the ten data from experimental batches were used to train the network for 150 epochs. During this period, the network repeatedly adjusted the synaptic weights towards reducing the prediction error. To validate the network, data from seven batches, both trained and untrained were presented to the network for prediction. There was a relatively acceptable correlation between the predicted output and the experimental output (Fig 5). It is believed that the predicted performance could be increased by selecting the training input data which are close to the input profile to be predicted using the minimum Euclid distance as earlier suggested by Horiuchi et al. [7].

Genetic algorithm search

With the set-up parameters (Fig 2), the GA search module iteratively evaluated the various temperature profiles in each generation using the trained neural network for profile evaluation and the predicted acidification slope as performance value. At each generation, the average performance as well as the performance of the best individual improved (Figs. 6, 7). A difference in the slope value of the best individual below a value of 0.0001 compared to the last generation was used as the stopping criterion. This was met at the 11th generation and the search stopped. The acidification slope improved from a value of 0.0127 to 0.06117. The evolution of the best profiles is shown in Fig 7. The evolved optimal temperature profile had a set point sequence of 43, 38, 44, 43, and 39 °C. This emphasizes the earlier experimental suggestion that process initialization at high temperature and termination at a slightly lower temperature results in a faster acidification. However, in the above profile, the sudden dropping of the temperature at 38 °C after 1 h of fermentation on the acidification metabolism of both organisms is not clearly understood, but it may be suggested that the application of a smoothing algorithm on this profile may generate a more rewarding industrial temperature profile. Laboratory evaluation of three replicates of the GA suggested optimum profile of 43, 38, 44, 43, and 39 °C gave an average slope of 0.04132.

Conclusion

The acidification behavior of *L. bulgaricus* and *S. thermophilus* in yogurt production across an optimal tempera-

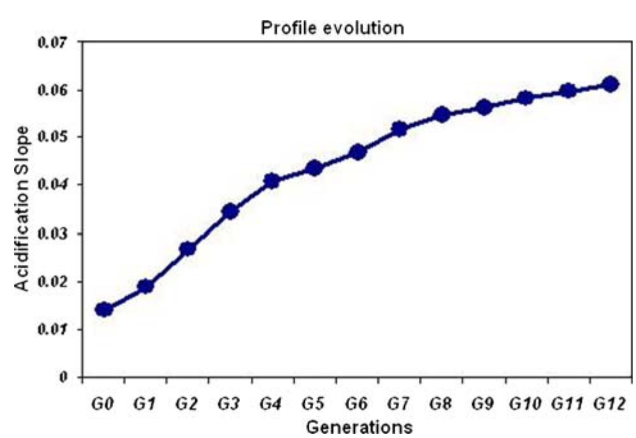


Fig. 6 Performance evolution of the genetic algorithm

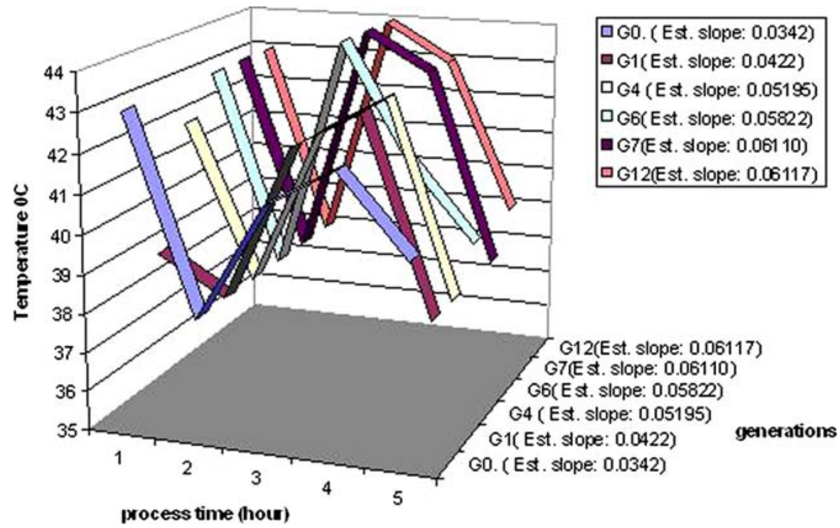


Fig. 7 Evolution of best profiles

ture window has been studied. Quick acidification using a programmed temperature stress profile has been elucidated. Our findings revealed that fast acidification could be achieved by initializing the fermentation process at 43 °C and terminating it at 39 °C. However, the interactive effect of the temperature stress for fast acidification and the metabolism of flavoring compounds which ultimately affect the taste and acceptability of the product have not been investigated. More so, traditional problems of model-plant mismatches should not be overlooked.

The profile optimization search engine software based on ANN and GA used in this work has proven to be effective in capturing the nonlinear behavior underlying the fermentation process contained in the training data, and so it iteratively evolved a temperature profile which could drive the process optimally. This module eases the determination of optimal control profiles of physico-chemical parameters of industrial fermentations without an in-depth knowledge of the process dynamics.

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