Mathematical Biosciences and Engineering

MBE, 19(10): 10687−10709.
DOI: 10.3934/mbe.2022500
Received: 28 March 2022
Revised: 11 July 2022
Accepted: 13 July 2022
Published: 27 July 2022

http://www.aimspress.com/journal/MBE

Research article

Online prediction of total sugar content and optimal control of glucose feed rate during chlortetracycline fermentation based on soft sensor modeling

Ping Wang[1], Qiaoyan Sun[1], Yuxin Qiao[1], Lili Liu[1], Xiang Han[2] and Xiangguang Chen[1,2,*]

[1] Department of Electrical and Electronic Engineering, College of Engineering, Yantai Nanshan University, Longkou 265713, China
[2] School of Chemistry and Chemical Engineering, Beijing Institute of Technology, Beijing 100081, China

* **Correspondence:** Email: xgc1@bit.edu.cn; Tel: +8613601333018; Fax: +8601068914662.

Abstract: In the process of chlortetracycline (CTC) fermentation, no instrument can be used to measure the total sugar content of the fermentation broth online due to its high viscosity and large amount of impurities, so it is difficult to realize the optimal control of glucose feed rate in the fermentation process. In order to solve this intractable problem, the relationship between on-line measurable parameters and total sugar content (One of the parameters that are difficult to measure online) in fermentation tank is deeply analyzed, and a new soft sensor model of total sugar content in fermentation tank and a new optimal control method of glucose feed rate are proposed in this paper. By selecting measurable variables of fermentation tank, determining different fermentation stages, constructing recursive fuzzy neural network (RFNN) and applying network rolling training method, an online soft sensor model of total sugar content is established. Based on the field multi-batch data, the change trend of the amount of glucose feed required at each fermentation stage is divided, and the online prediction of total sugar content and the optimal control strategy of glucose feed rate are realized by using the inference algorithm of expert experience regulation rules and soft sensor model of total sugar content. The experiment results in the real field demonstrate that the proposed scheme can effectively predict the total sugar content of fermentation broth online, optimize the control of glucose feed rate during fermentation process, reduce production cost and meet the requirements of production technology.

Keywords: chlortetracycline fermentation; total sugar content; soft sensor model; optimal control strategy; optimal control of glucose feed rate

1. Introduction

1.1. Chlortetracycline production technology

Chlortetracycline is a broad-spectrum tetracycline antibiotics and it is the final product of Streptomyces aureus through successive amplification. Chlortetracycline mixed into animal feed can be used as one of antibiotic products. Chlortetracycline has an inhibitory effect on gram-positive bacteria, gram-negative bacteria, spirochete, Rickettsiella, mycoplasma, chlamydia and other bacteria, and has the characteristics of bacteriostasis, high utilization rate, growth promotion, and less residue [1]. Therefore, for many years, chlortetracycline has been the largest dosage of bacteriostatic growth promoting agent in animal husbandry feed, has a wide range of uses in medicine, animal husbandry and other fields. Because chlortetracycline is a microbial fermentation process, its fermentation process has the characteristics of large lag, nonlinear, time varying and uncertainty.

Chlortetracycline production process can be summarized as follows: Streptomyces aureus strains need to undergo sand spore tube, mother inclined plane, daughter inclined plane, primary seed tank, secondary seed tank culture, and then transplanted to fermentation tank for cultivation. Before the strain is transplanted to the fermentation tank, the fermentation tank is sterilized (usually by high-pressure steam), and the culture medium is fed into the fermentation tank, followed by high temperature sterilization. Finally, 15% inoculation amount of Streptomyces aureus strain was transplanted into the fermentation tank. During fermentation, nutrients such as glucose substrate, pure soybean oil and peptone are supplemented for the metabolism of the strain. In the process of chlortetracycline fermentation, the pH value in the fermentation tank is adjusted by injecting ammonia, sterile air is pumped to regulate dissolved oxygen (DO), and cold or hot water is input to regulate temperature (FT), which will provide suitable conditions for the growth of bacterial strains. Chlortetracycline fermentation process is shown in Figure 1.

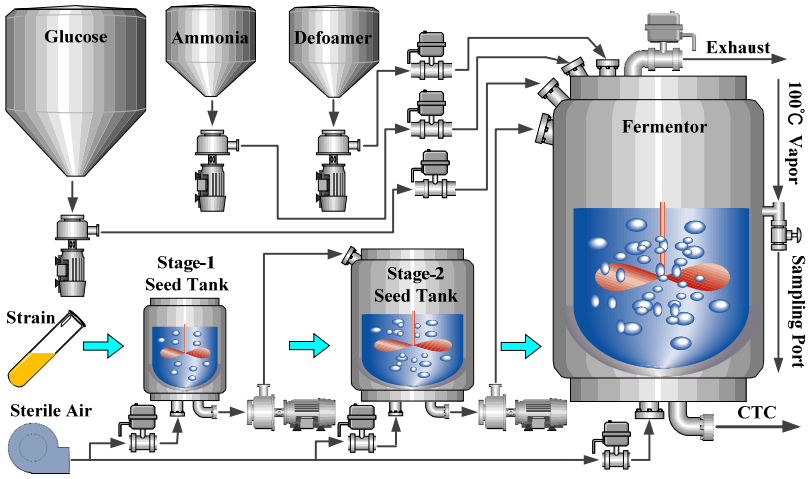


Figure 1. Schematic diagram of chlortetracycline fermentation process.

1.2. Total sugar content

The synthesis pathway of chlortetracycline is closely related to sugar metabolism. Figure 2 describes the pathway of chlortetracycline synthesis, and intermediates of sugar metabolism are the basis of chlortetracycline synthesis [2]. In the development phase of chlortetracycline bacteria, approaches of the metabolism of sugar for glycolysis, it is a glucose degradation into lytic enzyme process of pyruvic acid, and is accompanied by ATP (adenosine triphosphate), at the same time generate chlortetracycline bacteria related material, pyruvate metabolism will continue to generate intermediate for synthesis of chlortetracycline. In the production stage of chlortetracycline metabolism, Streptomyces aureus will follow a pentose metabolic pathway to produce NADPH (reduced coenzyme A), which is the reducing power energy of chlortetracycline synthesis, and the resulting pentose phosphate will participate in nucleic acid metabolism [3].

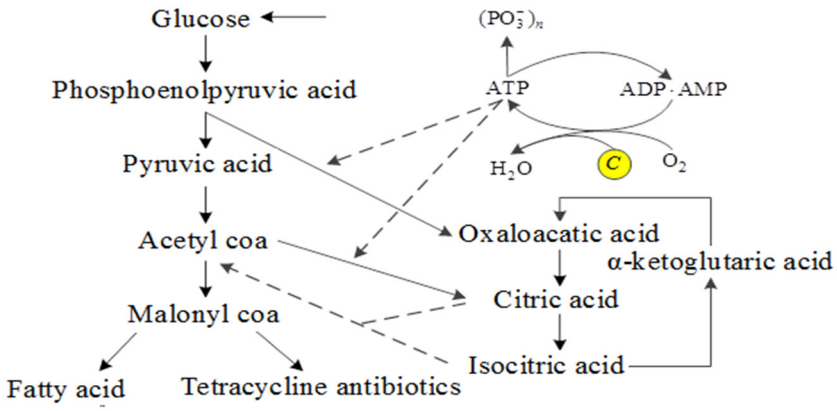


Figure 2. The relationship between chlortetracycline synthesis and glucose metabolism.

Based on the principle of biological metabolic balance, the metabolic reaction of chlortetracycline (CTC) in culture and fermentation can be described simply. Several major macro metabolic reactions are [4]: Reaction of bacterial cell growth, CTC synthesis, decomposition of sugar (substrate) and energy generation; The substances involved are mainly substrate sugar S, nitrogen (or ammonia), oxygen ,, as well as thistle X, product P (CTC) and CO2. Substances considered in the metabolic process are mainly NADH and ATP, which represent reducing power and energy. CTC synthesis pathway is shown in Figure 3.

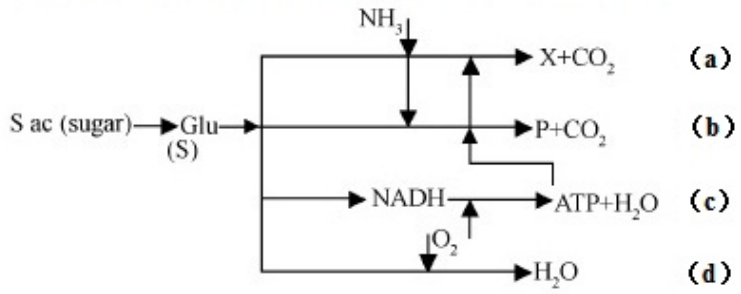


Figure 3. The scheme of metabolic pathways for chlortetracycline biosynthesis.

The total sugars mentioned in this paper include glucose, fructose, pentose, lactose, maltose, as well as starches that can be hydrolyzed. These sugars have reductive properties. In chlortetracycline fermentation, these sugars are collectively referred to as total sugars. The chemical determination of total sugars is time-consuming (And it is offline detection) and not suitable for on-line determination of industrial processes. In this paper, the total sugar content is detected by a test program called "Determination of the total sugar content of chlortetracycline fermentation broth by refractometer" (patent No. CN102297847A). This method is used for the manual determination of total sugar content mentioned in the following sections.

The importance of on-line detection of total sugar content: In the process of chlortetracycline fermentation, because the total sugar content of fermentation broth can not be detected online, it can only be completed by manual timing sampling analysis. Because there are multiple fermentation tanks on the production site, manual field sampling and then to the laboratory for analysis, this process is labor-intensive and time-consuming. At present, the sampling and analysis interval of chlortetracycline factories in China is set at 4−8 hours. In order to reduce the labor cost, the factory involved in this paper currently sets the interval of on-site sampling for off-line analysis as 8 hours. Due to the long time interval, the total sugar content in fermentation tank fluctuates greatly, and it is very difficult to adjust it online, which has a great impact on production indexes such as biological potency and fermentation volume (i.e., product quality and yield).

So far, pH, temperature, dissolved oxygen and pressure can be detected online and controlled automatically in the process of chlortetracycline fermentation, while the total sugar content is analyzed offline and the glucose feed process is controlled manually. The total sugar content is an on-line parameter that is difficult to measure (the fermentation broth is too viscous for the sensor to measure in direct contact). Therefore, the analyst first took samples on site and filtered them in the analysis room (as shown in Figure 4). Then the total sugar content value was obtained through instrumental analysis. This process takes several hours to complete.

Figure 4. Chlortetracycline fermentation broth (1 represents the fermentation broth in the fermentation tank, and 2 represents the filtered solution).

1.3. Review of previous work

Total sugar includes reducing sugar and non-reducing sugar. Reducing sugar is an active carbohydrate and can be quantitatively detected by direct chemical titration, spectrophotometry, high performance liquid chromatography, infrared spectroscopy and other methods [5]. In order to achieve rapid nondestructive detection of LBP and total sugar content by hyperspectral image technology, H. Yu et al. processed original hyperspectral images by principal component analysis and established prediction models based on different characteristic parameters by partial least squares regression. Experimental results show that, in order to improve the accuracy of prediction results, all features are screened according to the correlation degree of relevant indicators, and the detection model constructed by optimized features has good prediction results [6]. The total sugar content in edible fungi was detected by multi-microporous plate detector. The experimental results show that the results obtained by the multi-porous plate detector are in good agreement with those obtained by the national standard method, but the new method used by the author consumes less reagents, has fast detection speed and good reproducibility [7]. The total sugar content in edible fungi is an important quality index. The Chinese standard (GB/T 15672-2009) method for the determination of total sugar content in edible fungi is spectrophotometric method. The shortcomings of this method are slow detection speed, consumption of reagents.

At present, there are some methods to determine the total sugar content, such as DNS method [8], conductivity method [9], method of infrared spectroscopy [10], refraction method [11], etc. The analysis instrument used in manual analysis in this paper is based on the measuring principle of refraction method. Firstly, sample (fermentation broth) is taken from the on-site fermenting tank, then filtered by filter paper, and then filtered liquid is dropped onto the measuring mirror of the analysis instrument to display the value of total sugar content [12]. Cozzolino et al. analyzed the fermentation process of wild yeast using refraction principle [13]. The ATAGO RX-7000i analyzer (made in Japan) is in this paper used for off-line analysis of total sugar content in the analysis laboratory.

Y. Li [14] describes the reaction process in the fermentation tank, and introduces oxygen supply and demand and transfer, microbial fermentation mechanism, fermentation kinetics, biological reactor, fermentation process control and prevention of bacterial contamination. The main purpose of K. H. Petie and P. Trayana [15] is to design the feasibility of online prediction of process variables (such as concentrations of lysine, glucose and amino nitrogen, etc.) in the lysine fermentation process based on artificial neural network. T. Damak [16] proposed a method of estimating biological variables based on data through an example of a nonlinear distributed parameter bioreactor. Simulation results show that the estimator can be used for nonlinear estimation and robust processes. M. R. García et al. [17] proposed a method for measuring oxygen concentration in fermentation tank based on basic biochemical principles. The method was applied to a horizontal tubular reactor in which free-growth aspergillus produced gluconic acid. In order to study the corresponding relationship between the total sugar content of Junzao jujube in Southern Xinjiang and hyperspectral data, K. Surisetty et al. [18] used hyperspectral imaging technology combined with genetic algorithm and back propagation neural network (BP network) to model and analyze the total sugar content of Junzao jujube in Southern Xinjiang. The original spectral data were processed by different pretreatment methods and the wavelength variables were selected by genetic algorithm. The experimental results show that MSC is better than other pretreatment methods. The BP neural network was better than partial least squares (PLS) in predicting the total sugar content of Junzizao in southern Xinjiang. For the parameter

identification of fermentation process, L. Chen and F. Liu [19] established the model principle of each operation point of fermentation process based on the least square method, and obtained multiple models at different points. The total or global model is then calculated using a weighting function or interpolation method. J. Deng et al. [20] is to apply data-driven model and model update strategy to soft measurement, and use data-driven model to estimate parameters under the framework of expectation maximization (EM) algorithm. The soft measurement method is applied to predict online quality variables of industrial processes. A nonlinear model predictive controller (NMPC) is proposed to determine the optimal substrate feed flow for Escherichia coli culture on an experimental device [21]. Since biological process models are often uncertain, an NMPC optimization scheme is proposed and simulated using Monte Carlo analysis method. The research team of Professor Jian-lin Wang from Beijing University of Chemical Engineering has made great progress in the research and application of soft sensor modeling methods [22−24]. In recent years, our research team has undertaken several research projects on Chlortetracycline fermentation process, and has published some relevant articles in Chinese and English journals [25−32].

1.4. The organizational structure of this paper

The rest of this paper is structured as follows. Section 2 provides soft sensor modeling method of total sugar content, including methods for determining the fermentation stages of chlortetracycline, online rolling prediction method of total sugar content in fermentation tank, and recurrent fuzzy neural networks (RFNN) structure. Section 3 proposes a method for optimal control of glucose feed rate in chlortetracycline fermentation process, including expert experience regulation rule reasoning and optimal control algorithm of glucose feed rate. Section 4 discusses an application Example in the real field. Section 5 summarizes the research work and gives some innovations.

2. Modeling method of soft sensor of total sugar content

In this section, we will propose a soft sensor modeling method for total sugar content in chlortetracycline fermentation process, which includes dividing several stages of chlortetracycline fermentation process by total sugar consumption, online rolling prediction method for total sugar content and improved RFNN network structure.

2.1. Determination method of chlortetracycline fermentation stage

Chlortetracycline is the final product of Streptomyces aureus through successive amplification, culture and fermentation. A batch of chlortetracycline fermentation lasts about 85−120 hours. According to the different consumption of total sugar during the strains growth of fermentation tank, the fermentation process can be divided into four stages, that is, delay period, exponential growth period, stable period and decline period. The main characteristics of each growing period are as follows:
1) Delay period: the growth of bacteria is slow, the consumption rate of nutrients (i.e., total sugar consumption) is small, and the fermentation time is between 0 and t1.
2) Exponential growth stage: the bacteria grow exponentially, the consumption rate of nutrients (i.e., total sugar consumption) increases sharply, and the fermentation time is between t1 and t2.
3) Stable period: The growth of the bacteria slows down, the concentration of the bacteria is stable, the

formation rate of the product reaches the maximum, the consumption rate of nutrients (i.e., total sugar consumption) is relatively high, and the fermentation time is about between t2 and t3.
4) Decay stage: the bacteria begin to autolysis, the fermentation process is coming to an end, and the fermentation time is between t3 and t4.

According to the different feeding operation modes, the feeding operation of chlortetracycline fermentation can be divided into two modes:
1) In the delay period, a certain amount of nutrients are input to the fermentation tank at the beginning of fermentation, and there is no need to carry out feeding operation in the fermentation process. Therefore, this mode of operation is not usually adopted in large-scale production processes.
2) After the exponential growth period begins, the fermentation tank needs to be continuously supplemented with nutrients (i.e., continuous feeding fermentation production process).

In this paper, based on the statistical analysis of the historical data of the fermentation tank and the experience of the field engineers and operators, a method is proposed to divide the fermentation stages based on the glucose feed rate (i.e., the rate of consumption of total sugar). It can be divided into four stages: stagnation stage, growth stage, stable stage and decline stage. Its operation mode is shown in Figure 5. In the figure, t is the fermentation time, and q is the consumption rate of total sugar in the fermentation process.

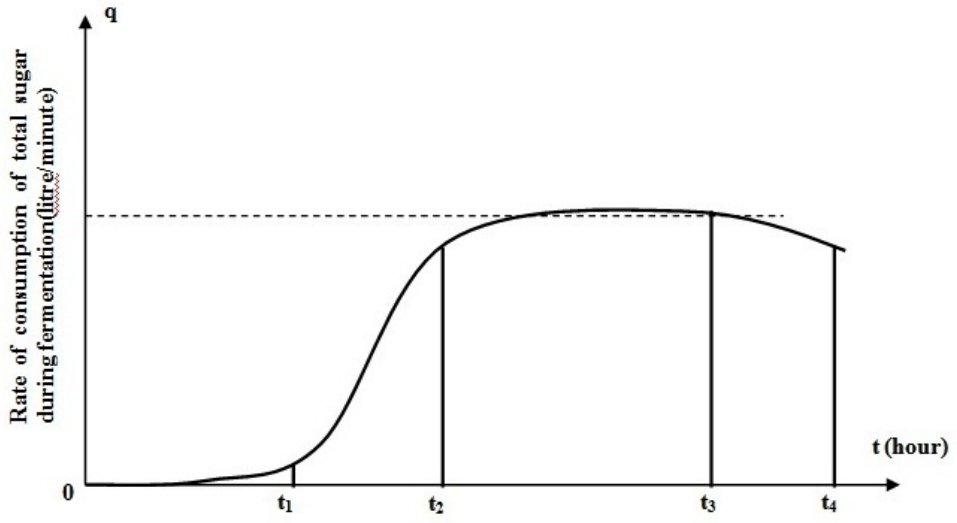


Figure 5. The fermentation stages of the fermentation process are divided based on the consumption rate of total sugar.

2.2. Online rolling prediction method of total sugar content in fermentation tank

The online rolling prediction method of total sugar content in fermentation tank is shown in Figure 6, and its basic principle is explained as follows:

In the process of chlortetracycline fermentation, due to the total sugar content can not be detected online, manual timing to the site sampling, and then back to the laboratory for analysis. In order to reduce labor costs, manual sampling interval of chlortetracycline fermentation tank in China is currently set at 4−8 hours. In order to reduce the cost of labor, the two chlortetracycline factories we

studied set the interval of manual sampling and analysis at 8 hours (because several parameters, such as total sugar content, amino nitrogen, biological potency, etc., need to be analyzed in one sample). When the time interval is long, the total sugar content of fermentation tank fluctuates greatly, which has a great influence on the production indexes such as biological potency and fermentation volume (i.e., product quality and yield).

As shown in Figure 6, three soft sensor values (i.e., predicted values of total sugar content) are evenly inserted into the interval of 8-hour offline sampling analysis values. The input values of the prediction model include not only offline analysis value and online predicted value in this sampling period, but also offline analysis value and soft sensor predicted value in the previous sampling period. Different from soft sensor values in the usual sense, this paper takes several historical process values and current state values as the input of the model. By giving them different influence weights, the results of soft sensor can not only eliminate the random disturbance of input data, but also reflect the change of total sugar content after 2 hours of fermentation. The accuracy of soft sensor can be improved obviously by using this method.

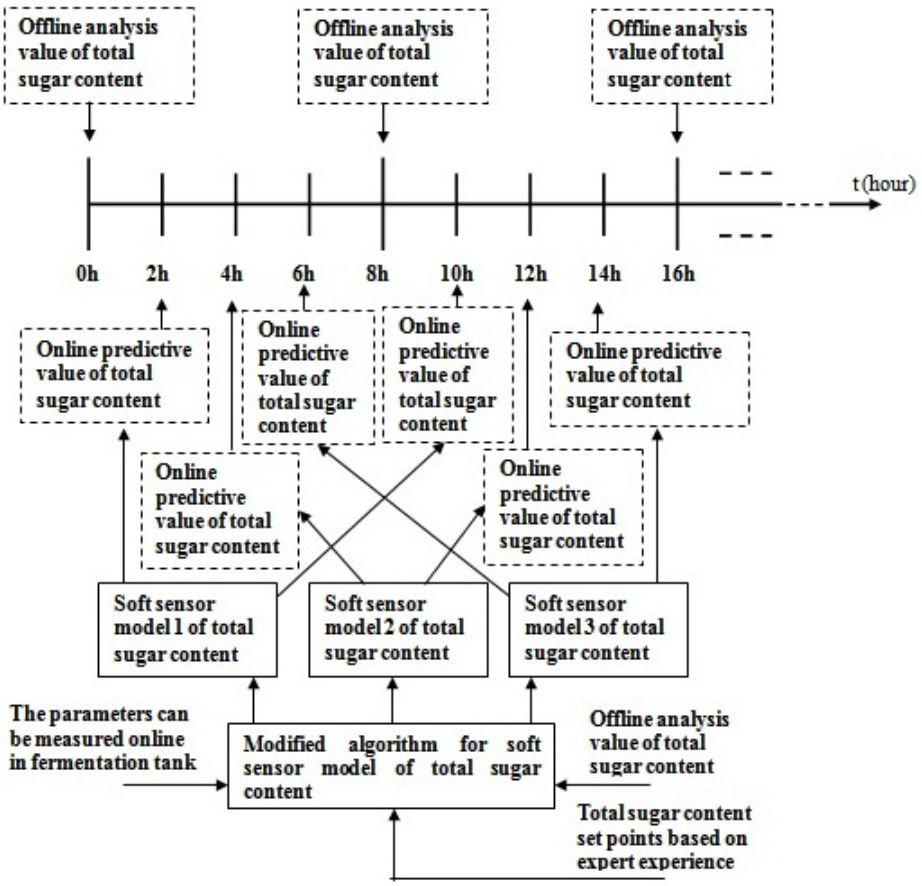


Figure 6. Online rolling prediction method of total sugar content in fermentation tank.

2.3. Improved recurrent fuzzy neural networks (RFNN) structure

In this paper, the improved RFNN network structure is used to construct the soft sensor model, as shown in Figure 7.

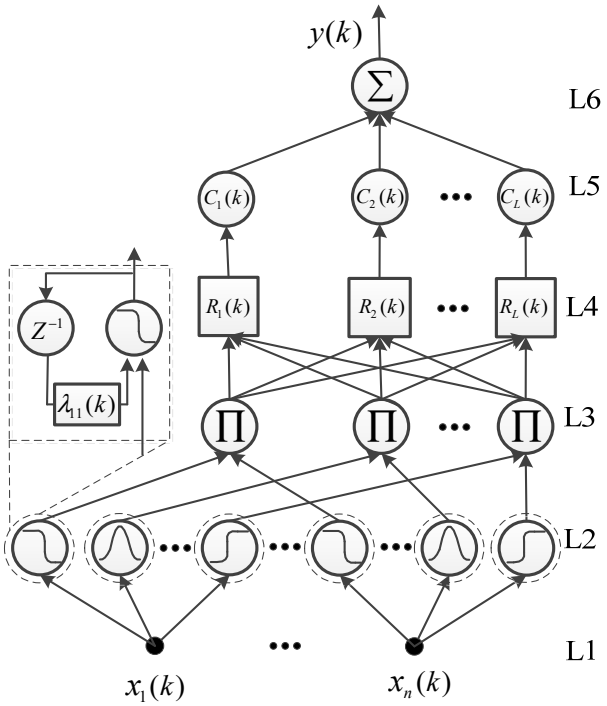


Figure 7. Structure diagram of recursive fuzzy neural network (RFNN).

The network structure in Figure 7 contains *n* input nodes and one output node, where *k* represents discrete time series values. In the structure in Figure 7, the first layer receives all input variables, and the second layer fuzzies the input variables. The number of fuzzy membership functions, the initial center value and width value of the membership function are the data field clustering analysis values of the input variables.

Each node in this layer stores the membership value of the previous round of calculation, so the output value of the network node can be calculated as

$$\mu_{il}(k) = exp\left(-\frac{(x_i(k)+\mu_{il}(k-1)\lambda_1-\theta_{il})^2}{2\sigma_{il}^2}\right) \tag{1}$$

Where, i represents the *i*th input variable, $i = 1, 2, \ldots, n$. l represents the *l*th fuzzy rule, $l = 1, 2, \ldots, m$. θ_{il} and σ_{il} are the center value and width value of the Gaussian membership function respectively. Each node in the third layer performs an "and" calculation on the input value of the previous layer, $\bar{\mu}_l(k) = \prod_{i=1}^{n} \mu_{il}\,(k)$. The output of the node at the fourth layer represents the ratio between the influence degree of rule l and the influence degree sum of all rules. $\omega_l(k) = \frac{\bar{\mu}_l}{\sum_{l=1}^{m} \bar{\mu}_l(k)}$. Where, $0 \le \omega_l(k) \le 1$, and $\sum_{l=1}^{m} \omega_l(k) = 1$. The fifth layer is the output weight of rule l multiplied by the network output value after RFNN. $\omega_l(k)(\sum_{i=0}^{n} p_{il}x_i(k)) = \omega_l(k)\bar{y}_l(k)$. The sixth layer is the defuzzification layer. $y(k) = \sum_{l=1}^{m} \omega_l\, \bar{y}_l(k)$. The calculation equations for updating RFNN network parameters θ_{il}, σ_{il}, λ_{il} and p_{il} are Eqs (2)−(5).

$$\theta_{il}(k+1) = \theta_{il}(k) + \eta(y(k) - \bar{y}(k))(\bar{y}_l(k)\ldots - \bar{y}(k))\omega_l(k)\frac{(x_i(k)+\mu_{il}(k-1)\lambda_{il}-\theta_{il})}{\sigma_{il}^2} \tag{2}$$

$$\sigma_{il}(k+1) = \sigma_{il}(k) + \eta(y(k) - \bar{y}(k))(\bar{y}_l(k)\ldots - \bar{y}(k))\omega_l(k)\frac{(x_i(k)+\mu_{il}(k-1)\lambda_{il}-\theta_{il})^2}{\sigma_{il}^3} \tag{3}$$

$$\lambda_{il}(k+1) = \lambda_{il}(k) + \eta\big(y(k) - \bar{y}(k)\big)\ldots\big(\bar{y}_l(k) - \bar{y}(k)\big)\omega_l(k)\ldots$$

$$\frac{(p_{il}(k)-x_i(k)-\mu_{il}(k-1)\lambda_{il})^2}{\sigma_{il}^2}\mu_{il}(k-1) \tag{4}$$

$$p_{il}(k+1) = p_{il}(k) + \eta(y(k)\ldots - \bar{y}(k))\omega_l(k)x_i(k) \tag{5}$$

Note: Figure 7 is a general structure diagram, so the expression of output parameter in Figure 7 (such as $c(k)$, $w(k)$, $z(k)$, etc.) can be rewritten according to the actual application.

2.4. Selection of modeling auxiliary variables

Currently, in the process of chlortetracycline fermentation, the process variables that can be measured online include fermentation tank temperature (TE), fermentation tank pressure (TP), pH value (pH), mixer rate (MR), dissolved oxygen (DO), accumulation of defoaming agents (AD), fermentation broth volume (FV), ammonia accumulation (AA), accumulation of sugar supplementation (AS), motor current (MC), fermentation time (FT) and the relative gas content in fermentation tank exhaust gas.

Because the fermentation tank outlet exhaust gas has viscosity and contains impurities, it is easy to make analytical pipeline jam in measuring instruments. The error of on-line measurement of carbon dioxide content and oxygen content in fermentation tank exhaust gas by current analytical instruments on the market is very large.

In this paper, AA is the accumulated amount of ammonia water. When the pH value in the fermentation tank fluctuates, the pH value can be changed within the range required by the set value by adjusting the flow rate of ammonia water. MR is the stirring rate of the stirrer in fermentation tank, which has a certain influence on the dissolved oxygen (DO) of the fermentation tank and the change of related parameters. AD is the accumulated amount of defoamer because the fermentation tank produces bubbles during stirring that will carry the broth out of the fermentation tank if they are not removed. AS is the accumulated amount of supplementary sugar. During the fermentation process, nutrients (such as glucose or hydrolyzed starch, etc.) should be continuously supplied to the required bacteria in the fermentation tank.

The correlation coefficient of multiple independent variables and one dependent variable is calculated as follows:

$$r(x_{i,j}^k, c_i^k) = \frac{Cov(x_{i,j}^k, c_i^k)}{\sqrt{Var[x_{i,j}^k]Var[c_i^k]}} \tag{6}$$

Where, $Cov(x_{i,j}^k, c_i^k)$ is the covariance of $x_{i,j}^k$ and c_i^k, $Var[x_{i,j}^k]$ is the variance of $x_{i,j}^k$, and $Var[c_i^k]$ is the variance of c_i^k. $i = 1, 2, \ldots, N,\ j = 1, 2, \ldots, M,\ k = 1, 2, \ldots, K$.

k represents the serial number of a batch data set of T306 fermentation tank, i represents the serial number of independent and dependent variables of a group of data in T306 fermentation tank, j

represents the serial number of variables that can be measured online in T306 fermentation tank.

$k = 1, 2, \ldots, K$, $i = 1, 2, \ldots, N$; $j = 1, 2, \ldots, M$. K is the number of T306 fermentation tank batches, M is the number of parameters that the fermentation tank can measure online and $K$is the total number of batches of T306 fermentation tank, M is the number of parameters that T306 fermentation tank can be measured online, and N is the number of data sets of each batch of T306 fermentation tank.

50 batches of sample data were selected for the T306 fermentation tank experiments, Using typical variable correlation analysis method, the average correlation degree between measurable process variables and total sugar c was calculated as follows:

$$\bar{r}_{i,j} = \frac{1}{K}\sum_{i=1}^{K} r(x_{i,j}^{k}, c_i^{k}) \tag{7}$$

The results are shown in Table 1.

Table 1. Correlation between process measurable variables and total sugar content.

Process variables	The correlation between the process variable and Cy $\bar{r}_{i,j}$
TE	0.26
TP	0.04
pH	0.41
MR	0.72
DO	0.92
AD	0.76
FV	0.13
AA	0.76
AS	0.94
MC	0.71
TF	0.71

From the Table 1, select the process variables with $\bar{r}_{i,j} > 0.7$ as the modeling auxiliary variables, then the model input variables are MR, DO, AD, AA, AS, MC, TF. The total sugar content (Cy) is set as the output variable of the model.

3. Optimal control method of total sugar consumption during chlortetracycline fermentation

In the present section we discuss the optimal control method of glucose feed rate in the process of chlortetracycline fermentation, including the optimal control system structure diagram of glucose feed rate optimal control algorithm and regulation rules.

3.1. Optimal control system of glucose feed rate in fermentation tank

In this paper, an optimal control scheme of glucose feed rate during chlortetracycline fermentation is proposed, and its control system structure is shown in Figure 8. The control system consists of seven parts, Namely chlortetracycline fermentation tank (B1) and the parameters of the fermentation tank can be on-line detected (B2), total sugar content in offline analysis values (B3), online soft sensor model of total sugar content (B4) and expert experience regulation and inference rules and

modification model of control algorithm parameters (B5), optimization control algorithm of glucose feed in chlortetracycline fermentation tank (B6), and actuator of glucose feed (B7). The main functions of each component of the system are described as follows:

1) Chlortetracycline fermentation tank (B1) is the controlled object of the control system, and its applicable volume is 50−200 m^3 fermentation tank. The volume of fermentation tank in this paper is 120 m^3.

2) The fermentation tank can detect parameters online (B2), including temperature, pressure, pH, DO, carbon dioxide content and oxygen content at the outlet of the fermentation tank, etc.

3) The off-line analysis value of total sugar content (B3) refers to the sampling analysis value between 2 and 10 hours. The analyst takes samples from the on-site fermentation tank and goes back to the analysis laboratory for off-line analysis to obtain the total sugar content value at the sampling time.

4) Online soft measurement model of total sugar content (B4), based on offline analysis value of total sugar content (B3) and online detection value of fermentation tank parameters (B2), the online prediction value $C_{\hat{y}}$ of total sugar content in fermentation tank is realized.

5) The modified model (B5) of expert experience regulation and inference rules and control algorithm parameters is based on the online detection parameter (B2) of fermentation tank, the offline analysis value of total sugar content (B3), the output value of (B4) and the output value of (B6) $U(t)$, creating conditions for the modified strategy of (B6).

6) Optimization control algorithm (B6) of glucose feed in chlortetracycline fermentation tank is based on field data, fluctuation of raw material ingredients and expert experience regulation rules to determine the control algorithm, in order to realize the optimal control of glucose feed rate.

7) Actuator of glucose feed (B7) is a control device for glucose feed in fermentation tank.

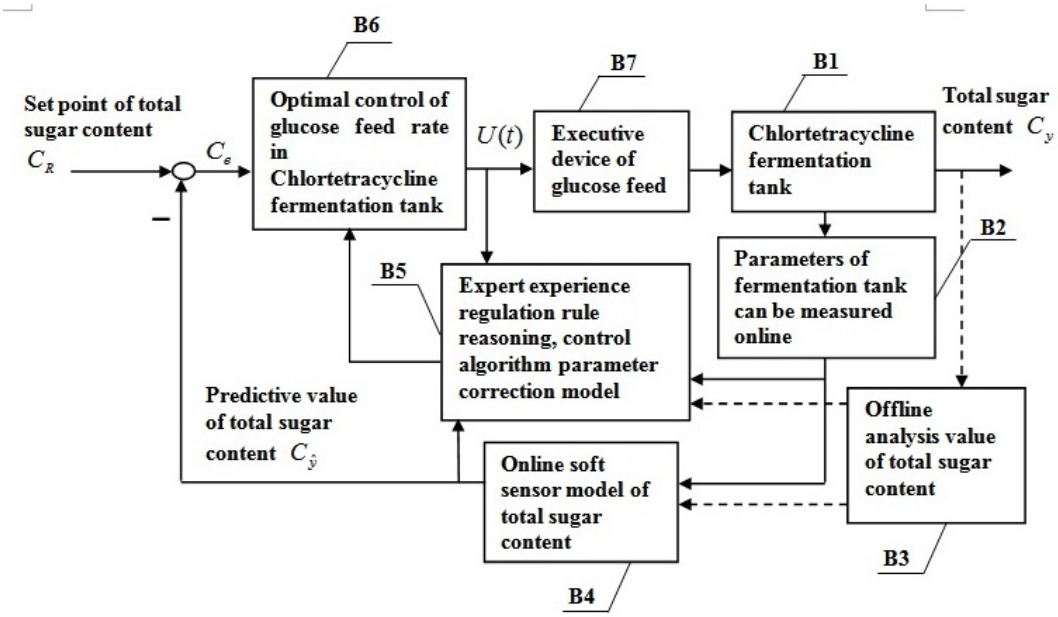


Figure 8. Structure diagram of optimal control system for glucose feed rate in fermentation tank. Note: C_y and $C_{\hat{y}}$ in Figure 8 are equivalent to c and $\hat{c}$ in the text.

3.2. Optimal control algorithm of glucose feed rate and regulation rules

According to the deviation of the artificial analysis value of total sugar content from the set value, the adjustment formulas of glucose feed rate in the fermentation tank are as follows.

$$U(t) = U^*(t) + \Delta U(t) = U^*(t) + \delta(t)\left[\frac{\Delta C(t)}{Q_S(t)}\right] Q_F(t) \tag{8}$$

$$\delta(t) = f(k(t)) \tag{9}$$

$$k(t) = \frac{\Delta C(t)}{Q_S(t)} \tag{10}$$

$$\Delta C(t) = C_R(t) - C(t) \tag{11}$$

Where, t is the fermentation time; $U(t)$ is the glucose feed rate at time t; $U^*(t)$ is the set glucose feed rate; $C(t)$ is the total sugar content, $C_R(t)$ is the set value of total sugar content; $Q_S(t)$ is the adjustable range of total sugar content at time *t*; $Q_F(t)$ is the standard deviation of glucose feed rate. $k(t)$ is the deviation coefficient of total sugar content; $\delta(t)$ is the adjustment coefficient of glucose feed rate.

The total sugar deviation coefficient $k(t)$ was determined based on the change of the total sugar content in the fermentation tank, and the adjustment coefficient $\delta(t)$ is determined by the total sugar deviation coefficient $k(t)$. Based on the field values and expert operating experience, the inference rules for the adjustment coefficient of glucose feed rate are obtained, as shown in Table 2.

Table 2. Determining the adjustment coefficient $\delta(t)$ of sugar supplementation rate based on the variation range of $k(t)$.

Rules	Based on the range of $k(t)$	Determining the adjustment coefficient $\delta(t)$
Rule 1	$0.7 < k(t) \leq 1$	$\delta(t)$ = 0.5
Rule 2	$1 < k(t) \leq 1.3$	$\delta(t)$ = 0.8
Rule 3	$1.3 < k(t) \leq 1.5$	$\delta(t)$ = 0.9
Rule 4	$k(t) > 1.5$	$\delta(t)$ = 1
Rule 5	$-1 \leq k(t) < -0.7$	$\delta(t)$ = -0.5
Rule 6	$-1.3 \leq k(t) < -1$	$\delta(t)$ = -0.7
Rule 7	$-1.5 \leq k(t) < -1.3$	$\delta(t)$ = -0.8
Rule 8	$k(t) < -1.5$	$\delta(t)$ = -0.9
Rule 9	$-0.7 \leq k(t) \leq 0.7$	$\delta(t)$ = 0

4. Field experiment and discussion

Chlortetracycline fermentation is a multi-stage intermittent production process, and the biochemical activities of growth, reproduction, metabolism and synthesis of Streptomyces aureus are also different in different fermentation stages. Therefore, corresponding control schemes for different fermentation stages need to be implemented.

Chlortetracycline fermentation can be divided into four stages based on the total sugar consumption of the fermentation tank. When the total sugar content in the fermentation broth is lower than or higher than the expected total sugar content of the four stages (that is, it exceeds the set upper or lower limit value), it is necessary to adopt the effective strategy of glucose feed in time and use the optimized feeding method to add glucose and other nutrients into the fermentation tank within the set sampling interval.

4.1. Hardware composition and software design of the experimental system

Hardware composition of the experimental system for optimal control of glucose feed rate in Chlortetracycline (CTC) fermentation process is shown in Figure 9. In order not to affect the production site, the experimental system and the field application DCS system is run in parallel. The software design of the experimental system is developed by using LabVIEW and adopts modular design scheme. The software design framework of the system is shown in Figure 10. In Figure 10, T is the sampling period, D is the total time of batch fermentation process, T301–T309 are numbers for 9 fermentation tanks (120 m^3).

Figure 9. Optimal control experimental system of glucose feed rate in Chlortetracycline (CTC) fermentation process.

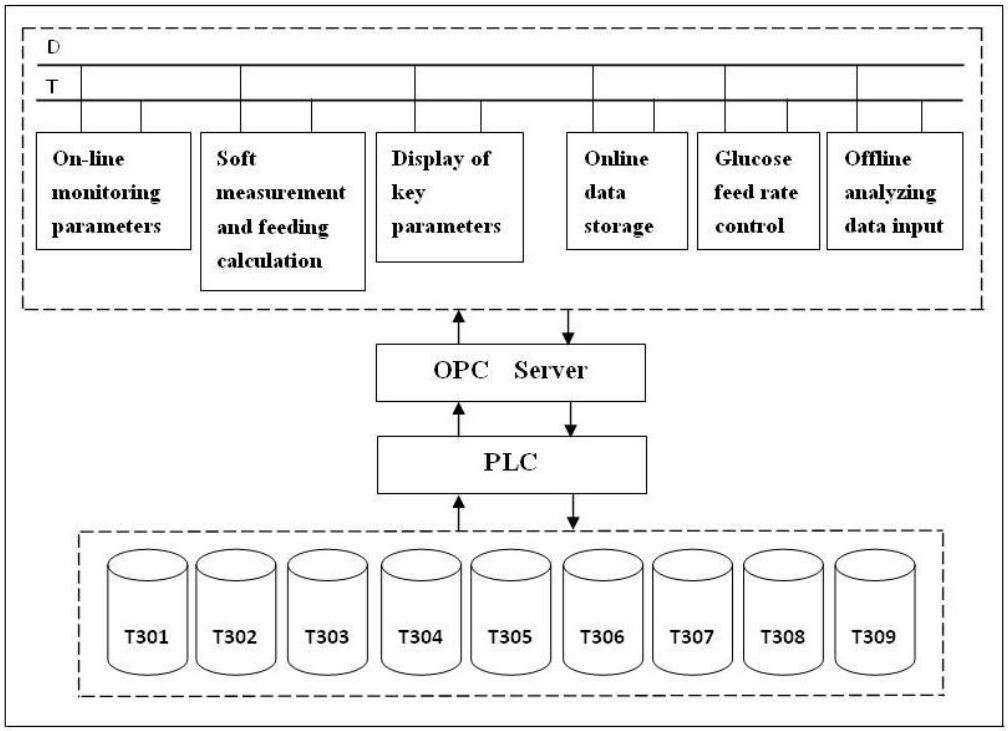


Figure 10. Experimental framework of optimal control of glucose feed rate in chlortetracycline (CTC) fermentation process.

4.2. The importance of sugar supplement in chlortetracycline fermentation process

For example, in a certain fermentation stage, if the amount of sugar supplement can not meet the growth demand of the fermentation tank bacteria, then the bacteria will be in a state of hunger without carbon source, which will inhibit the reproduction and growth of the bacteria, and the phenomenon of autolysis will occur in the fermentation process. On the contrary, if the amount of sugar is too high, the bacteria will be in a state of high sugar content, which will make the bacteria overgrow and reproduce, and consume a large amount of nutritional supplements, which is not conducive to the synthesis of chlortetracycline.

4.3. Comparison between offline analysis and online prediction of total sugar content

Because there are about 20−30 fermentation tanks (these include several primary seed tanks, several secondary seed tanks and a number of fermentation tanks) in the whole chlortetracycline process, samples of each fermentation tank need to be analyzed 3−5 parameters (Such as total sugar content, amino nitrogen, biological potency, etc.). Considering production and labor costs, at present, Chinese factories set the sampling interval of chlortetracycline fermentation tank at 6−8 hours.

The comparison between offline analysis and online prediction of total sugar content needs to be verified by multiple batches of experimental data. Field engineers and workers are satisfied with the online prediction results of total sugar content. The comparison results are shown in Figures 11 and 12.

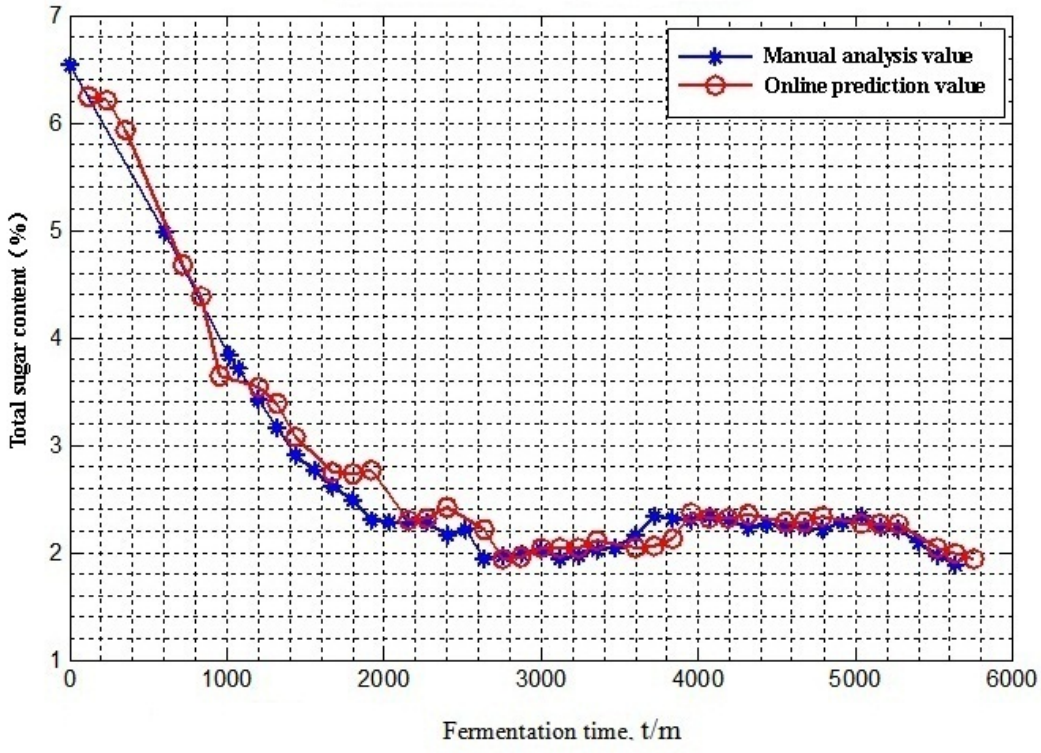


Figure 11. Comparison between offline analysis and online prediction of total sugar content in a batch of T306 fermentation tank.

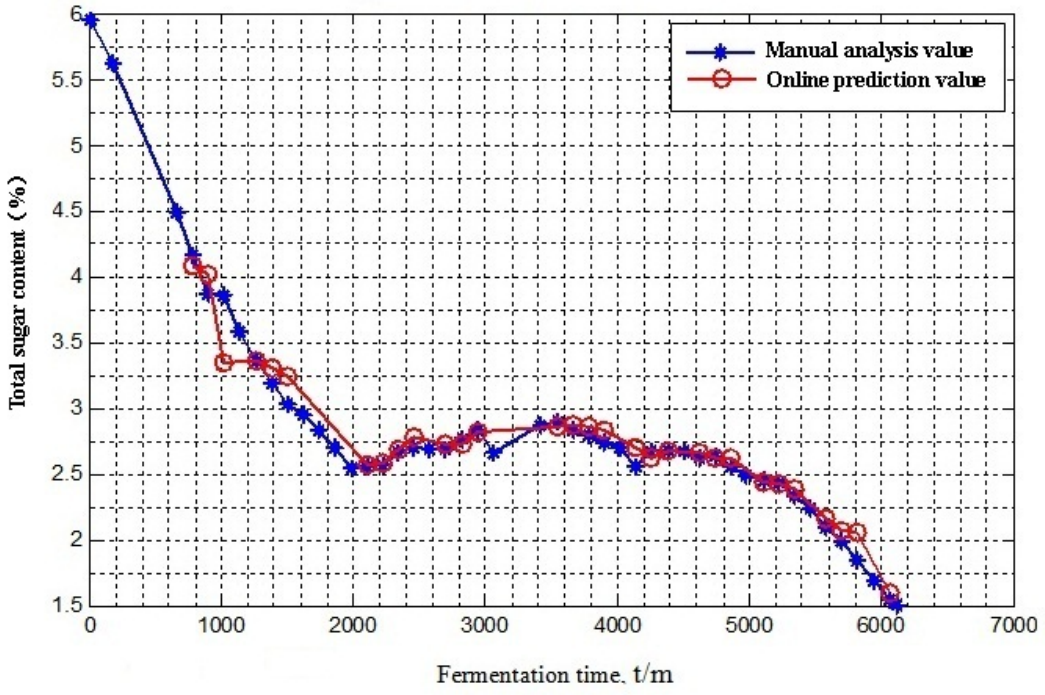


Figure 12. Comparison between offline analysis and online prediction of total sugar content in another batch of T306 fermentation tank.

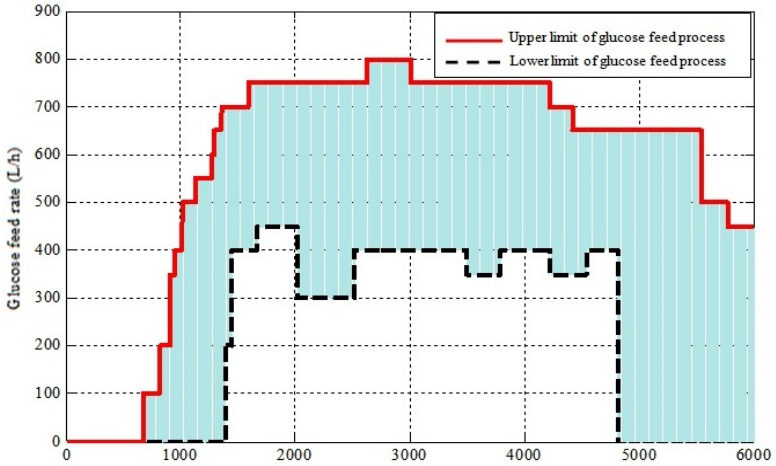
Offline measurements of total sugar content are: First, the analyst takes samples from the production site, and then goes back to the laboratory to filter the fermentation broth. Then, the total sugar content is measured by measuring instrument. This process is called off-line measurement of total sugar content. The current total sugar content of the fermentation tank is used to determine the glucose feed rate. The artificial adjustment of sugar supplementing rate is based on the analysis value of total sugar content, the operator input corresponding glucose feed rate value on the industrial control computer. The analyst inputs the manual analysis value on the computer in the analysis laboratory, and the input data will be automatically entered into the measurement and control system database.

Online predictions of total sugar content are: 1) MATLAB was used to establish the prediction model, and the prediction model was trained based on the historical production data on site; 2) The T306 fermentation tank was designated as the main experimental device in this research project, and the factory leader specially arranged analysts to assist in the verification of the prediction model. It took several months (i.e., multiple batches of fermentation data) to verify the accuracy of the prediction model.

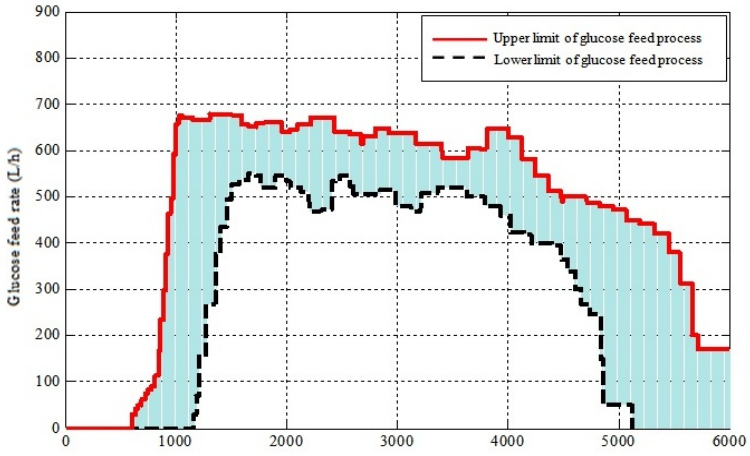
4.4. Comparison between artificially adjusted and optimized glucose feed rate

It can be seen from (a)−(c) in Figure 13 that artificial adjustment of glucose feed rate is based on the experience and judgment of operators, and each operator has some differences in adjustment methods and experience. This will lead to a wide range of fluctuation in the adjustment process of glucose feed rate, which will also cause the instability of the fermentation process, and will not only increase the production cost, but also affect the yield and quality of Chlortetracycline products; When the soft sensor model is used to predict the total sugar content and optimize the glucose feed rate, the fluctuation range of the regulation process is relatively small, which made the fermentation process relatively stable. This not only reduces the production cost, but also improves the yield and quality of Chlortetracycline products.

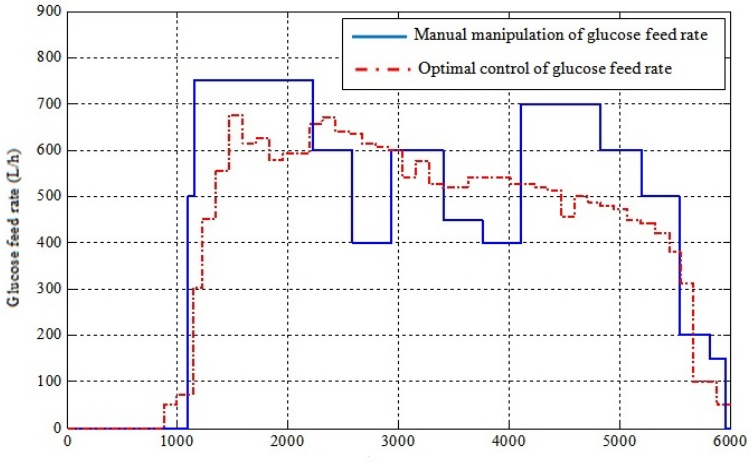
In Figure 13, (a)−(c) are the comparison charts of fluctuation range of glucose feed rate corresponding to the two different regulation methods and the comparison charts of transition curve of glucose feed rate corresponding to the two different regulation methods. As shown in Figure 13(a),(b), the statistical average fluctuation range of artificial adjustment of glucose feed rate is 362 L/h, while the statistical average fluctuation range of optimal control is 189 L/h.



(a) Chart of fluctuation range of manual glucose feed process



(b) Fluctuation range diagram of automatic glucose feed process



(c) The comparison diagram of glucose feed process of two glucose feed methods in a batch

Figure 13. The fluctuation range comparison of two adjustment methods for glucose feed rates ((a) manual operation, (b) Optimal control).

In the process of chlortetracycline fermentation, in addition to some variables (such as pH value, DO, temperature, etc.) that can be measured online, there are also several variables (such as total sugar content, amino nitrogen, biological potency, etc.) that are difficult to be measured online. In order to realize the optimal control of chlortetracycline fermentation process, it is necessary to have a method to predict the variables that are difficult to measure online. The content of this paper is online prediction of total sugar content and optimal control of glucose feed rate. Based on the prediction of online total sugar content, the optimal control of glucose feed rate can be realized, so as to achieve the goal of stable fermentation process, reducing the total sugar supplementing (that is, reducing the production cost) and improving the product quality.

i) The set values of glucose feed rate are the regulation rules summarized by field engineers and operators after years of practice, but the regulation rules should also be changed according to the analysis values of the total sugar content of the fermentation tank. As the interval time for artificial analysis of total sugar content is set as 6−8 hours, the adjustment of glucose feed rate would be delayed greatly. ii) The soft sensor model is used to predict the total sugar content online (The predicted time interval is 2 hours), and the glucose feed rate of fermentation tank could be adjusted automatically. See Figures 11 and 12. iii) Firstly, the on-site operators can adjust the glucose feed rate according to the total sugar content value through sampling, filtering and measurement, which is the artificial adjustment process of glucose feed rate. iv) The first part of this paper focuses on building a soft sensor model to make an online prediction of the total sugar content of the fermentation tank. The second part focuses on the optimal control of glucose feed rate of fermentation tank, so as to reduce production cost, stabilize production process and improve product quality.

As can be seen from Figure 13: 1) due to the large lag time of artificial analysis of total sugar content, the control of glucose feed rate is not timely and fluctuated greatly; 2) The prediction model based on the total sugar content can control the rate of glucose feed in time with small fluctuation, reduce the total amount of glucose feed and reduce the production cost. The superposition of the two curves in Figure 13 is intended to facilitate comparison and analysis.

In this section we will be considering the following factors for performing experiments on the production site.

i) When we do experiments on the production site, we must not affect the normal production process in order to avoid economic losses or casualties to the production plant.

ii) When the experimental process needs to obtain online measurement data from the production process monitoring center or database, the factory leaders and related engineers should be contacted in advance, and the field experiment can only be carried out after obtaining formal permission and commitment.

Note: The online soft sensor model of total sugar content established in this paper has been run in the field for several months and compared with the manual analysis value. The predicted value of the model can only be used as the measurement value of total sugar content in the optimal control system of glucose feed rate when the measurement accuracy is satisfied and the operator's approval is obtained. In order not to affect the production process, the validation process of the prediction model takes several months, which requires multiple batches of data to be validated.

5. Conclusions

In the process of chlortetracycline fermentation, because the fermentation broth contains a lot of

impurities, and its viscosity is very large. At present, no instrument can be used to measure the total sugar content of fermentation broth online, so it is difficult to realize the optimal control of glucose feed rate in fermentation process. In order to solve this challenging problem, the authors first established the online soft sensor model of total sugar content, and then proposed the expert experience regulation rules and inference algorithm. The optimal control of glucose feed rate in fermentation process is realized. The innovations of this paper can be summarized as follows:

1) Several different fermentation stages are divided based on the changing trend of the amount of supplemental sugar required in the chlortetracycline fermentation process.

2) Based on the measurable variables of fermentation tank, a recursive fuzzy neural network (RFNN) is constructed and a network rolling training method is used to establish an online soft sensor model of total sugar content in the fermentation tank.

3) The online prediction of total sugar content and the optimal control of glucose feed rate are realized by the expert experience regulation rules and the corresponding inference algorithm.

4) The experimental results show that the online prediction model of total sugar content and the optimal control strategy of sugar supplement rate proposed in this paper have the advantages of reducing production cost and improving the yield and quality of chlortetracycline production. Therefore, the online soft-sensing model and optimized control scheme can also be applied to related industrial production processes (or biochemical production processes).

Acknowledgments

This scientific work is financially supported by Yantai "Double Hundred Plan" Talent Project (YT201803) in 2018，Natural Science Foundation (No. ZR2016FM28) of Shandong Province in 2016. We also thank Charoen Pokphand Group for their financial support and for providing the industrial datasets offed-batch CTC fermentation process.

Conflict of interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

References

1. T. Wu, X. Yang, S. Li, Application of chlortetracycline in animal husbandry (in Chinese), *Sichuan Anim. Vet. Sci.*, **42** (2015), 35−36. Available from: https://www.docin.com/p-1559269672.html.
2. A. L. Cinquina, F. Longo, G. Anastasi, L. Giannetti, R. Cozzani, Validation of a high-performance liquid chromatography method for the determination of oxytetracycline, tetracycline, chlortetracycline and doxycycline in bovine milk and muscle, *J. Chromatogr. A*, **987** (2003), 227−233. https://doi.org/10.1016/S0021-9673(02)01446-2
3. J. Zhu, D. D. Snow, D. A. Cassada, S. J. Monson, R. F. Spalding, Analysis of oxytetracycline, tetracycline, and chlortetracycline in water using solid-phase extraction and liquid chromatography–tandem mass spectrometry, *J. Chromatogr. A*, **928** (2001), 177−186. https://doi.org/10.1016/S0021-9673(01)01139-6

4. J. L. Fu, Y. P. Zhuang, M. Z. Huang, J. Chu, S. L. Zhang, Application of element and metablism balancing for the cultivation process with Streptomyces aureofaciens, *Chin. J. Biotechnol.*, **19** (2003), 471−475. Available from: https://cjb.ijournals.cn/cjbcn/article/abstract/03040471.
5. X. Hu, Research progress in the detection and analysis of total and reducing sugars in food (in Chinese), *Shandong Chem. Ind.*, **50** (2021), 99−100. https://doi.org/10.19319/j.cnki.issn.1008021x.2021.19.037
6. H. Yu, R. Wang, Y. Yin, Y. Liu, Prediction of polysaccharide and total sugar in wolfberry based on spectral and texture information from hyperspectral image (in Chinese), *J. Nucl. Agric. Sci.*, **32** (2018), 523−531. https://doi.org/10.11869/j.issn.100-8551.2018.03.0523
7. S. Yuan, Y. Shang, Y. Qiu, A new method on determination of the total sugar content in edible fungi (in Chinese), *Sci. Technol. Food Ind.*, **34** (2013), 78−79. https://doi.org/10.13386/j.issn1002-0306.2013.18.044
8. X. Qi, X. Teng, J. Zuo, S. Li. Determination of reducing sugar in citric acid fermentation broth (in Chinese), *Farm Mach.*, **8** (2011), 141−144. https://doi.org/10.16167/j.cnki.1000-9868.2011.23.019
9. L. Chen, M. Cheng, X. Xiao, Z. Huang, Measurement of the relationship between conductivity of salt solution and concentration and temperature (in Chinese), *Res. Explor. Lab.*, **29** (2010), 39−42. Available from: https://oversea.cnki.net/kcms/detail/detail.aspx?dbcode=CJFD&dbname=CJFD2010&filename=SYSY201005013.
10. F. Shen, Y. Ying, Q. Sun, J. Hu, Determination of total sugar content in Chinese rice wine by mid-infrared spectroscopy (in Chinese), *Trans. Chin. Soc. Agric. Mach.*, **40** (2009), 143−145. Available from: https://www.docin.com/p-804072569.html.
11. S. Jiang, Q. Yang, *Fermentation Analysis and Detection Technology (in Chinese)*, Chemical Industry Press, Beijing, China, 2008. Available from: http://www.cip.com.cn/Home/Contact.
12. H. Liu, *Development of the Online Automatic Measuring System for Total Sugar Content in Chlortetracycline Fermenter (in Chinese)*, Master's thesis, Chemical Engineering and the Environment, Beijing Institute of Technology, Beijing, China, 2013. Available from: http://lib.bit.edu.cn/node/404.jspx.
13. D. Cozzolino, C. Curtin, The use of attenuated total reflectance as tool to monitor the time course of fermentation in wild ferments, *Food Control*, **26** (2012), 241−246. https://doi.org/10.1016/j.foodcont.2012.02.006
14. Y. Li, *Principle and Technology of Fermentation Engineering (in Chinese)*, Higher Education Press, Beijing, China, 2007. Available from: https://www.hep.com.cn/.
15. K. H. Petie, P. Trayana, Neural network software sensors design for lysine fermentation process, *Appl. Artif. Intell.*, **22** (2008), 235−253. https://doi.org/10.1080/08839510701881458
16. T. Damak, Procedure for asymptotic state and parameter estimation of nonlinear distributed parameter bioreactor, *Appl. Math. Modell.*, **31** (2007), 1293−1307. https://doi.org/10.1016/j.apm.2006.02.014
17. M. R. García, C. Vilas, E. Balsa-Canto, V. N. Lyubenova, M. N. Ignatova, A. A. Alonso, On-line estimation in a distributed parameter bioreactor: application to the gluconic acid production, *Comput. Chem. Eng.*, **35** (2011), 84−91. https://doi.org/10.1016/j.compchemeng.2010.05.005

18. K. Surisetty, H. De laHoz Siegler, W. C. McCaffrey, A. Ben-Zvi, Robust modeling of a microalgal heterotrophic fed-batch bioreactor, *Chem. Eng. Sci.*, **65** (2010), 5402−5410. https://doi.org/10.1016/j.ces.2010.06.008
19. L. Chen, F. Liu, Recursive parameter identification for fermentation processes with the multiple model technique, *Appl. Math. Modell.*, **36** (2012), 2275−2285. https://doi.org/10.1016/j.apm.2011.08.021
20. J. Deng, L. Xie, L. Chen, S. Khatibisepehr, B. Huang, F. Xu, et al., Development and industrial application of soft sensors with on-line Bayesian model updating strategy, *J. Process Control*, **23** (2013), 317−325. https://doi.org/10.1016/j.jprocont.2012.12.008
21. L. O. Santos, L. Dewasme, D. Coutinho, A. Vande Wouwer, Nonlinear model predictive control of fed-batch cultures of micro-organisms exhibiting overflow metabolism: assessment and robustness, *Comput. Chem. Eng.*, **39** (2012), 143−151. https://doi.org/10.1016/j.compchemeng.2011.12.010
22. T. Yu, J. Wang, K. He, L. Zhao, Staged soft-sensor modeling method for fermentation process based on MPCA-GP (in Chinese), *Chin. J. Sci. Instrum.*, **34** (2013), 2703−2708. Available from: https://www.wdfxw.net/doc95063421.htm.
23. K. He, L. Zhao, J. Wang, T. Yu, Soft-sensor modeling method in a fermentation process based on the samples of a sparse Gaussian process (in Chinese), *J. Beijing Univ. Chem. Technol.*, **41** (2014), 108−113. Available from: https://journal.buct.edu.cn/EN/Y2014/V41/I3/108.
24. Y. Gao, J. Wang, T. Yu, L. Zhao, A non-parametric soft sensor modeling method for use in fermentation processes (in Chinese), *J. Beijing Univ. Chem. Technol.*, **43** (2016), 74−78. Available from: https://journal.buct.edu.cn/EN/10.13543/j.bhxbzr.2016.05.013.
25. J. Yang X. Chen, H. Jin, Online prediction of contamination of chlortetracycline fermentation based on Dezert-Smarandache theory, *Chin. J. Chem. Eng.*, **23** (2015), 1009−1016. https://doi.org/10.1016/j.cjche.2014.06.043
26. H. Jin, X. Chen, J. Yang, L. Wu, Adaptive soft sensor modeling framework based on just-in-time learning and partial least squares regression for batch bioprocesses, *Comput. Chem. Eng.*, **71** (2014), 77–93. https://doi.org/10.1016/j.compchemeng.2014.07.014
27. H. Jin, X. Chen, J. Yang, L. Wu, L. Wang, Hybrid intelligent control of substrate feeding for industrial fed-batch chlortetracycline fermentation process, *ISA Trans.*, **53** (2014), 1822–1837. https://doi.org/10.1016/j.isatra.2014.08.015
28. J. Yang, X. Chen, H. Jin, L. Wu, Study on the industrial chlortetracycline fermentator glucose feed rate adjustment method based on soft sensor (in Chinese), *Chin. J. Sci. Instrum.*, **35** (2014), 468−474. Available from: https://www.wdfxw.net/doc85344352.htm.
29. J. Yang, X. Chen, H. Jin, L. Wu, Study on optimal scheduling strategy of CTC fermentation (in Chinese), *Trans. Beijing Inst. Technol.*, **34** (2014), 876−880. Available from: http://journal.bit.edu.cn/zr/article/id/20140820
30. Y. Sun, Q. Sun, X. Chen, N. Du, J. Yang, Research and application of amino nitrogen content soft sensor modeling method in the process of chlortetracycline fermentation, *J. Nanoelectron. Optoelectron.*, **14** (2019), 371−381. https://doi.org/10.1166/jno.2019.2545
31. Y. Sun, L. Tang, Q. Sun, M. Wang, X. Han, X. Chen, Study on the prediction of the contamination symptoms in the fermentation process of Chlortetracycline based on soft sensor modeling method, *Technol. Health Care*, **27** (2019), s205–s215. https://doi.org/10.3233/THC-199020

32. M. Wang, X. Han, Y. Sun, Q. Sun, X. Chen, Study on soft sensor modeling method for sign of contaminated fermentation broth in chlortetracycline fermentation process, *Prep. Biochem. Biotechnol.*, **51** (2021), 76−85. https://doi.org/10.1080/10826068.2020.1793173

AIMS Press