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Optimization of rice wine fermentation process based on the simultaneous saccharification and fermentation kinetic model☆

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ABSTRACT

Chinese rice wine making is a typical simultaneous saccharification and fermentation (SSF) process. During the fermentation process, temperature is one of the key parameters which decide the quality of Chinese rice wine. To optimize the SSF process for Chinese rice wine brewing, the effects of temperature on the kinetic parameters of yeast growth and ethanol production at various temperatures were determined in batch cultures using a mathematical model. The kinetic parameters as a function of temperature were evaluated using the software Origin8.0. Combining these functions with the mathematical model, an appropriate form of the model equations for the SSF considering the effects of temperature were developed. The kinetic parameters were found to fit the experimental data satisfactorily with the developed temperature-dependent model. The temperature profile for maximizing the ethanol production for rice wine fermentation was determined by genetic algorithm. The optimum temperature profile began at a low temperature of 26 °C up to 30 h. The operating temperature increased rapidly to 31.9 °C, and then decreased slowly to 18 °C at 65 h. Thereafter, the temperature was maintained at 18 °C until the end of fermentation. A maximum ethanol production of 89.3 g·L⁻¹ was attained. Conceivably, our model would facilitate the improvement of Chinese rice wine production at the industrial scale.

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1. Introduction

Chinese rice wine is a non-distillation beverage which is quite popular in south China [1]. So far, it is clear that temperature control in the Chinese rice wine brewing process is the most important controlling parameters governing the quality of Chinese rice wine [2]. The temperature during Chinese rice wine fermentation affects the following subprocesses [3]: (1) Cell growth/death. During the first 20 h, temperature affects cell growth and cell concentration affects ethanol production. (2) Starch hydrolysis. Temperature also affects the hydrolysis of starch from the sticky rice. (3) Fermentation rate of ethanol production. Temperature affects cell growth, particularly for the *S. cerevisiae* which transfers glucose to ethanol as we reported previously [2]. (4) Production of byproducts. Temperature affects the bacteria growth which produces organic acids.

However, the temperature control can be manually adjusted but it is necessary to determine the optimal temperature profile at various stages of the Chinese rice wine fermentation process. To determine the optimal temperature profile, the development of the kinetic model for the fermentation process is needed [4,5]. Thus far, although several mechanistic models have been proposed for enzymatic hydrolysis of cellulose [6,7], ethanol fermentation by *S. cerevisiae* [8], the optimum temperature for enzymatic hydrolysis of cellulose (typically 45–50 °C) is higher than that of the Chinese rice wine fermentation (30–35 °C) with *S. cerevisiae* as the fermenting organism. However, during the ethanol production, the enzyme for their study was purified cellulase and the temperature reached as high as 50 °C. In addition, most of their substrates were water-insoluble cellulose or starch flour. It is obviously different from that for Chinese rice wine fermentation. During the Chinese rice wine fermentation process, the temperature cannot exceed 35 °C, and the saccharification materials are whole steamed sticky rice. In addition, Chinese wheat *qu* instead of purify enzymes were used in the process [4,9,10]. All of the above indicate that Chinese rice wine fermentation is a new type SSF fermentation different from former ethanol fermentation. Consequently, although there have several model-based control of temperature during simultaneous saccharification and fermentation (SSF) has been experimentally determined for industrial ethanol fermentation; new kinetic model suitable for Chinese rice

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wine fermentation and the optimal temperature profile is still needed to be developed.

Hitherto, there are three methods to construct kinetic model: (1) Mechanism Model; (2) Data-driven model and (3) Gray-box model. Recently, our group has conducted a series of work to develop a suitable kinetic model for the Chinese rice wine fermentation based on the characteristics of Chinese rice wine brewing [11,12]. Both ethanol and organic acids in the Chinese rice wine can affect its quality. Acquiring high ethanol concentration and keeping the organic acids at suitable concentration are the main objectives for the process [13]. Like any other process, the basic challenge in Chinese rice wine brewing is to obtain high degree of hydrolysis of carbohydrate substrates together with high ethanol yield.

In this work, kinetic parameters were estimated from the kinetic model developed by our group, the temperature-dependent model has been developed and validated using the fermentation data with the industrial strain of the yeast *S. cerevisiae* Su-25 at various temperatures with pretreated whole stick rice. Subsequently, an optimal temperature profile for the SSF process of Chinese rice wine brewing to acquire high content of ethanol was determined based on the fitted model.

2. Materials and Methods

2.1. Mathematical model

The model includes temporal variations of the concentrations of starch (*S*), maltose (*M*), maltotriose (*R*), yeast cells (*C*), ethanol (*A*), dissolved oxygen (*O*), wheat *qu* (*E*), and starch-*qu* complex (*C*₁).

The mathematical model developed in this work was constructed based on our previous report [12]. As we describe in the kinetic model sections, the concentration of glucose were always low during the simultaneous saccharification and fermentation (SSF) process. At first, we hypothesized that the glucose was rapidly utilized by the yeast which was why the glucose level was always low. To verify our hypothesis, saccharification experiment was conducted. The result showed that the concentration of glucose was also as low as $3.5 \text{ g} \cdot \text{L}^{-1}$ in saccharification experiment only using wheat *qu* and starch, which was similar to that of under SSF condition. Therefore, during the Chinese rice wine fermentation process, maltose and maltotriose were the main sugar used by *S. cerevisiae* Su-25 to produce ethanol and other by-products.

Our mathematical model is shown as follows based our previous publication [12]:

$$\frac{dS}{dt} = -k_1SE \quad (1)$$

$$\frac{dR}{dt} = 1.037k_2C_1 - k_4 \frac{R}{K_{s1} + R} \frac{O}{K_{s2} + O} C \quad (2)$$

$$\frac{dM}{dt} = 1.056k_3C_1 - k_5 \frac{M}{K_{s3} + M} \frac{O}{K_{s2} + O} C - k_6 \frac{M}{K_{s4} + M} C \quad (3)$$

$$\frac{dC}{dt} = 0.084k_4 \frac{R}{K_{s1} + R} \frac{O}{K_{s2} + O} C + 0.088k_5 \frac{M}{K_{s3} + M} \frac{O}{K_{s2} + O} C \quad (4)$$

$$\frac{dA}{dt} = 0.54k_6 \frac{M}{K_{s4} + M} C \quad (5)$$

$$\frac{dO}{dt} = -k_4 \frac{R}{K_{s1} + R} \frac{O}{K_{s2} + O} C - k_5 \frac{M}{K_{s3} + M} \frac{O}{K_{s2} + O} C + O_{in} \quad (6)$$

$$\frac{dE}{dt} = -k_1SE + (k_2 + k_3)C_1 \quad (7)$$

$$\frac{dC_1}{dt} = k_1SE - (k_2 + k_3)C_1 \quad (8)$$

where the constants (1.037, 1.056, and 0.54) are the yield coefficients accounting for the molecular weight differences among the products; the other constants are obtained from the Balling equations [14]; K_{s1} – K_{s4} are the saturation constants of each substrate.

2.2. Materials

The Chinese wheat *qu* used in this study was supplied by the Shaoxing Nverhong Rice Wine Company (Zhejiang, China) and stored at room temperature. Sticky (glutinous) rice from northern China, was purchased from the Vanguard Market, Wuxi, China. Yeast strains *S. cerevisiae* Su-25 supplied by the Nverhong Wine Company (Shaoxing, Zhejiang, China) was used in this study and stored at 4 °C.

2.3. Thermo-controlled fermentation process

Rice wine fermentation experiments were carried out with rice wine yeast strains *S. cerevisiae* Su-25 and wheat *qu*.

The yeast strain was stored at 4 °C on slants of YPD agar medium. The yeast inoculum was transferred to a new slant of YPD agar and cultured for 24 h at 28 °C. A sloop of yeast culture was added to 50 ml YPD broth (250 ml flask) and cultured at 28 °C for 18 h. Based on the 1:10 ratio, yeast seed inoculum to new YPD broth (100 ml in 500 ml flask) and cultured at 28 °C for another 18 h.

The experiments were conducted in 7-L tank fermenters (BioFlow/CelliGen 115, New Brunswick Scientific, Enfield, CT, USA) containing about 5 L batch fermentation broth (1200 g steamed rice (dry weight), 204 g wheat *qu*, 120 ml yeast seed culture and 2400 ml tap water) at constant temperature for 96 h, four temperatures (18 °C, 23 °C, 28 °C, 33 °C) were chosen.

2.4. Analytical methods

At the predetermined time, samples of 2 ml cultures were taken from the fermentor for analyses with HPLC using the method describing by Liu *et al.* [3,11] as follows: 2 ml of samples from each experiment were taken out, and then centrifuged and filtered through 0.45-μm-pore Durapore (PVDF) membrane filters (Fisher, Pittsburgh, PA, USA), subsequently analyzed with high-performance liquid chromatography (HPLC). Agilent HPLC system (Agilent 1200 Series, Santa Clara, CA, USA) were used for injection and detection. An Aminex HPX-87H column (300 × 7.8 mm) (Bio-Rad Labs, Richmond, CA, USA) was used to identify and determine the levels of sugars (maltotriose, maltose and glucose) and ethanol with G1314B VWD detector and a G1362A RID detector (Agilent 1200 Series, Santa Clara, CA, USA). The HPLC conditions used were as follows: flow rate was $0.5 \text{ ml} \cdot \text{min}^{-1}$, the mobile phase consisted of 60-ml acetonitrile and 1270-μl sulfuric acid for every 1000-ml solvent, injection volume was 20 μl per fixed loop.

2.5. Determination of model parameters and its validation

To fit the data to the model, the parameters in the model were estimated using a nonlinear least square method which minimizes an objective function of *Z* [15]:

$$Z_X = \sum_{i=1}^n (X_{i,\text{exp}} - X_{i,\text{sim}})^2 \quad (9)$$

where *n* represents the number of data points, *Z_X*, represent the least squares discrepancies between experimental data and simulated results of biomass, *X* represent starch, maltose, yeast cells, ethanol, dissolved oxygen, wheat *qu*, and starch-enzyme complex, respectively. And *i* denotes the number of experimental data points. 'exp' and 'sim' denote the observed value, and simulated value, respectively. The model was constructed by using fourth-order Runge–Kutta method in MATLAB. MSE (mean squares error) and *R*² were used to evaluate the experimental

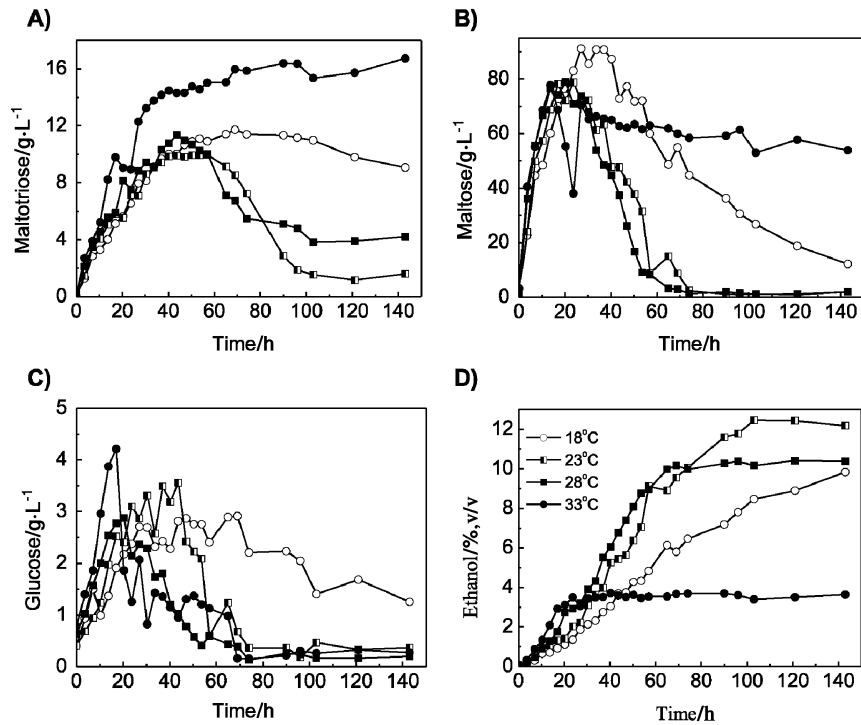


Fig. 1. The time course of the concentrations ($\text{g} \cdot \text{L}^{-1}$) of maltotriose, maltose, glucose, and ethanol at (A) 18 °C, (B) 23 °C, (C) 28 °C, (D) 33 °C of Chinese rice wine fermentation production.

data and modeling data. The MSE value can be calculated by the sum squares errors divided by the length of actual data period [16].

$$\text{MSE} = \frac{\sum_{i=1}^{NT} (y_i - f_i)^2}{n_t} \quad (10)$$

$$R^2 = 1 - \frac{\sum (y_i - f_i)^2}{\sum (y_i - \bar{y})^2} \quad (11)$$

where f_i , y_i , $\bar{y}$ and n_t are the model data, experimental data, mean experimental data and length of actual data period, respectively.

2.6. Development of temperature-dependent model and its validation

Kinetic parameters as a function of temperature in the range from 18 to 33 °C were estimated using the software package of Origin 8.0. These functions coupling with the fermentation kinetic model of Chinese rice wine fermentation were described in 2.1. A mathematical model was developed for the Chinese rice wine fermentation. This model considers the effects of temperature and it was verified with existing data.

2.7. Optimization of temperature profile

The optimal temperature profile for maximizing ethanol production was determined using genetic algorithms (GAs) with the developed model in Section 2.6. We pick the 'PopulationSize', 'Generations',

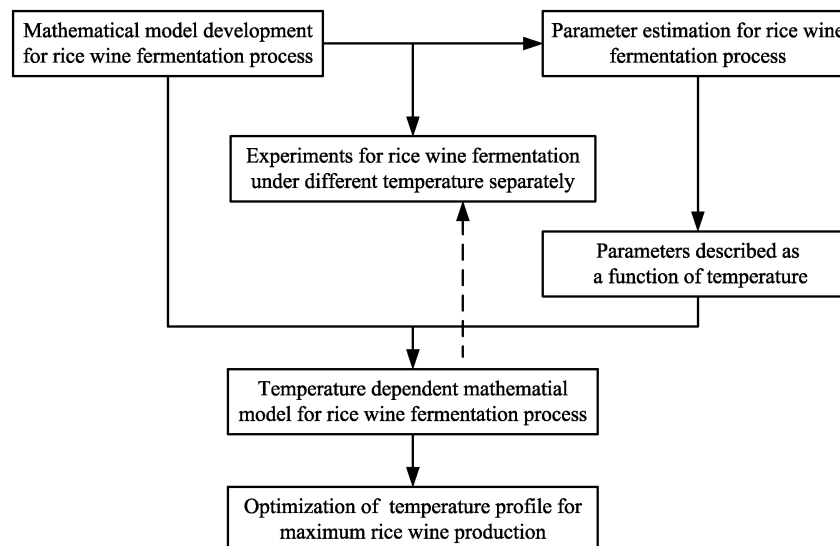


Fig. 2. Schematics for modeling and validating the temperature profile of the SSF process for Chinese rice wine.

Table 1

Estimated parameter values at various temperatures during Chinese wine fermentation

Temperature	k_1	k_2	k_3	k_4	k_5	k_6
18 °C	1.362	16.814	0.367	0.081	1.418	0.412
23 °C	1.623	19.812	0.591	0.562	2.631	0.812
28 °C	1.695	20.818	0.685	0.643	2.929	0.886
33 °C	2.594	20.810	1.334	0.164	2.946	1.462

Table 2Evaluation of the kinetic parameters as a function of temperature (T) during Chinese wine fermentation

Parameters	Function
k_1	$1.0608 \times 10^6 \exp\left[\frac{-33.018}{8.314 \times 10^{-3}} \left(\frac{1}{273+T} - \frac{1}{306}\right)\right]$
k_2	$-5.67058 + 1.79294 \times T - 0.03006 \times T^2$
k_3	$-0.00166 + 3.2 \times 10^{-4} \times T$
k_4	$-5.7482 + 0.4962 \times T - 0.0096 \times T^2$
k_5	$-7.41206 + 0.7076 \times T - 0.01196 \times T^2$
k_6	$4.85324 \times 10^9 \exp\left[\frac{-55.82684}{8.314 \times 10^{-3}} \left(\frac{1}{273+T} - \frac{1}{306}\right)\right]$

'StallTimeLimit', 'StallGenLimit', and 'TolFun', as 200, 100, Inf, 2, and 1×10^{-6} to run the GAs. The objective function was chosen as Eq. (11) for maximizing ethanol production by optimizing the temperature profile.

$$\min J = -A(t_f) \quad (12)$$

where $A(t_f)$ is the ethanol concentration at the end of fermentation.

3. Results and Discussion

3.1. Selection of key controlling variables and refinement of model

To construct model of the fermentation process and setup automatic control parameter, the most important procedure is determining the key controlling variables. For the Chinese rice wine production based on the China national standard GB-13662-2000 which can be classified into four types: dry-type (sugar content $\leq 15 \text{ g} \cdot \text{L}^{-1}$), half dry-type ($15 \text{ g} \cdot \text{L}^{-1} < \text{sugar content} \leq 40 \text{ g} \cdot \text{L}^{-1}$), half-sweet type ($40 \text{ g} \cdot \text{L}^{-1} < \text{sugar content} \leq 100 \text{ g} \cdot \text{L}^{-1}$) and sweet-type (sugar content $> 100 \text{ g} \cdot \text{L}^{-1}$).

To evaluate the effect of temperature on ethanol and sugar production, Chinese rice wine fermentation was carried out at 18 °C, 23 °C, 28 °C, and 33 °C. The results are shown in Fig. 1. The concentration of maltotriose at 33 °C increased gradually until fermentation end, and the concentration of maltotriose at 18 °C arrived at the highest concentration of $10.3 \text{ g} \cdot \text{L}^{-1}$ and then gradually reduced to $8.4 \text{ g} \cdot \text{L}^{-1}$ at 140 h. For the concentration of maltotriose at 28 and 23 °C, both of them all arrived at the highest concentration at $45 \text{ g} \cdot \text{L}^{-1}$ and following gradually reduced until 140 h (Fig. 1A). The concentration of maltose reached the highest capacity of $90 \text{ g} \cdot \text{L}^{-1}$ at 25 h and it remained unchanged until 40 h. Then, it began to reduce gradually. The concentration of maltose increased at the highest concentration of $79.5 \text{ g} \cdot \text{L}^{-1}$ at 25 h and then reduced quickly, and all arrived at lower values at 80 h. However, the concentration of maltose arrived at $80 \text{ g} \cdot \text{L}^{-1}$ at 20 h, it reduced to $40 \text{ g} \cdot \text{L}^{-1}$ at 25 h and then increased to $70 \text{ g} \cdot \text{L}^{-1}$, following reduced gradually until fermentation end (Fig. 1B). But the concentration of

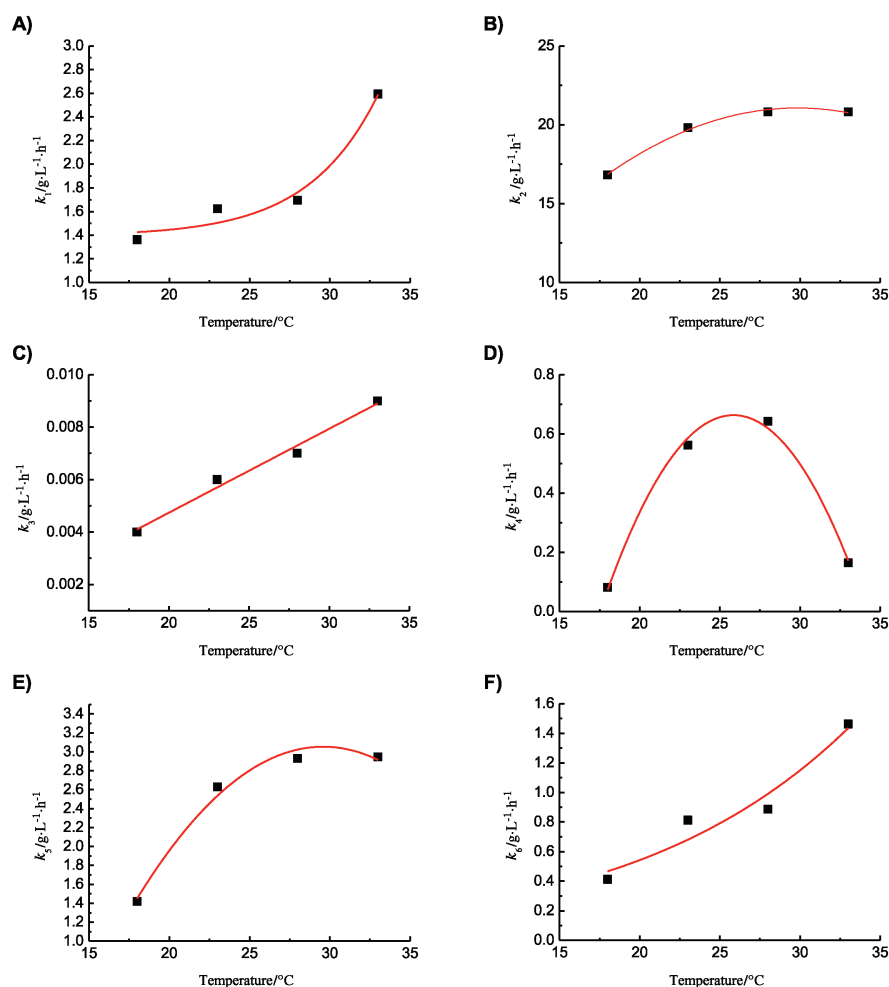


Fig. 3. Effect of temperature on the kinetic parameters (A) k_1 ; (B) k_2 ; (C) k_3 ; (D) k_4 ; (E) k_5 ; (F) k_6 during Chinese wine fermentation.

glucose only went up to around $3 \text{ g} \cdot \text{L}^{-1}$ (Fig. 1C). Consequently, maltose and maltotriose were chosen as variables but glucose was not considered in the resulting model.

For ethanol, from Fig. 1D, the final concentration of ethanol in Chinese rice wine broth increased with temperature increasing from 18 to 28 °C, and dropped to $30 \text{ g} \cdot \text{L}^{-1}$ at 33 °C. These results reveal that temperature control is an important parameter for ethanol production for Chinese rice wine brewing. Similar results were also reported for industrial ethanol fermentations [17,18]. Consequently, ethanol, maltose and maltotriose were considered as the major controlling parameters.

3.2. Development of the temperature-dependent model

To develop the model and validate the temperature profile for the SSF process, approaches described by Fig. 2 were used. The experimental data were utilized for parameter estimation with our accordingly developed kinetic model in this report. The estimated parameters from experimental data with our kinetic model are provided in Table 1. The parameter sensitivity test was conducted to examine the influence of the model parameters. Changes of $\pm 10\%$ were introduced to each parameter from its optimized value for the first ambient-temperature run and the ratio of the total sum of squared changes in the outputs (products) over the parameter change was computed as an indicator of the influence of the parameter or parameter sensitivity. The result revealed that k_1 , k_2 , k_3 , k_5 and k_6 are sensitive.

The temperature is an important parameter for ethanol fermentation. The reaction rate of enzyme for starch hydrolysis to produce maltose and maltotriose are also affected accordingly.

The effect of temperature on the specific hydrolysis rate of starch [19] and ethanol production [20] are quantitatively described by the Arrhenius equation which has been used to describe ethanol production with cellulose: $k_i(T_1) = k_i(T_2) \exp[-\Delta H / R(1/T_1 - 1/T_2)]$ in which $k_i(T_1)$ and $k_i(T_2)$ are the rate constant at the absolute temperature T_1 and T_2 ; R is the gas constant ($8.314 \times 10^{-3} \text{ kJ} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$); and ΔH is the

activation enthalpy of the reaction described by rate coefficient k_i ($\text{kJ} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$). We obtained ΔH of $33.018 (\text{kJ} \cdot \text{mol}^{-1} \cdot \text{K}^{-1})$ and $55.82684 (\text{kJ} \cdot \text{mol}^{-1} \cdot \text{K}^{-1})$ for k_1 and k_6 , estimated using Origin 8.0, respectively. Parameters k_2 , k_3 , k_4 and k_5 are used in model to describe maltotriose changing. Using the parameter values given in Table 1, kinetic parameters as a function of temperature were developed using Origin 8.0, the resulting model is shown in Table 2. Good agreement has been found between the developed function and the kinetic parameters (shown as Fig. 3).

Fig. 3 shows that k_1 and k_6 have the similar tendency within the temperature range 18–33 °C. The possible reason is that these parameters are used to describe fermentation kinetic with the Arrhenius equation and the reaction mechanism is the same. In addition, k_1 and k_6 , as well as, k_2 and k_5 have the same tendency. k_4 gradually increased and then decreased. And k_3 increased with the temperature increasing (Fig. 3).

3.3. Validation of the resulting temperature model with experimental data

After we developed the kinetic parameters as a function of temperature, the temperature-dependent model for describing the Chinese rice wine fermentation was worked out. However, to optimize the Chinese rice wine fermentation, we need to validate the model with the experimental data and the parameters summarized in Table 1.

Using the parameter function in Table 2, the data from the developed temperature-dependent model were shown as Fig. 4, in which indicates that a good agreement is found between the model and the experimental data for maltose and maltotriose concentrations, as well as ethanol production. In the Chinese rice wine fermentation broth, analysis of the cell concentration is still difficult. Therefore, in Fig. 4, the experimental data does not show the cell concentration. In addition, as Chinese rice wine is a semi-solid fermentation, the dissolved oxygen (DO) is difficult to determine accurately. The comparison of the ethanol concentrations of the experimental data and model at various temperatures are shown in Table 3. We concluded that all the

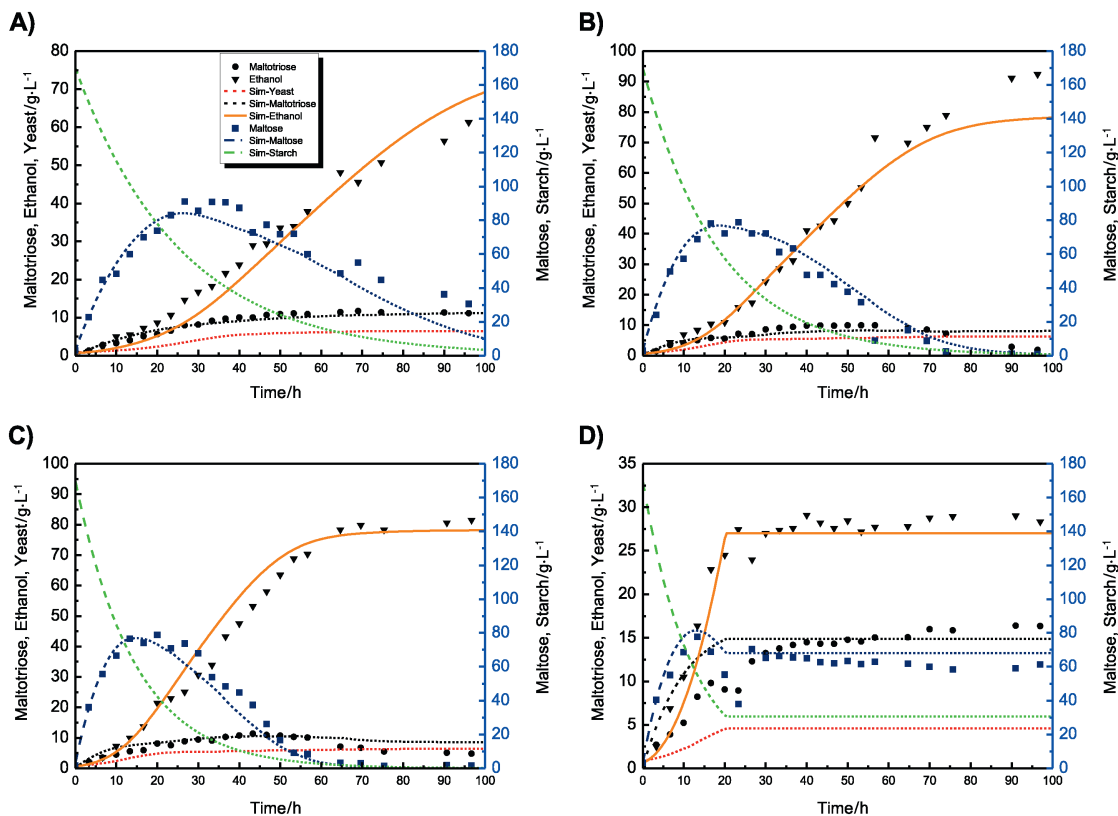


Fig. 4. Experimental data and model simulations for ethanol production with the development temperature-dependent kinetic model at various temperatures.

Table 3

The comparison of experimental and model predicted ethanol concentration for various temperatures

Temperature model/°C	Ethanol concentration at the end of 100 h/g·L ⁻¹	
	Model predicted	Experiment value
18	69.23	66.46
23	78.15	97.72
28	78.18	79.78
33	27.01	26.63

parameters of the developed model in this report are applicable to the SSF process besides the values at 23 °C. It is clear that the experimental data on ethanol level are higher than that of model predicted. The reason is that yeast growth is more robust at 23 °C, and has longer viability time at 23 °C than other temperatures. Consequently, higher ethanol level is produced by the yeast at 23 °C.

3.4. Optimization of the temperature for maximizing ethanol production

Rice wine fermentation is a typical nonlinear system. Genetic algorithm is effective for complex nonlinear system optimization. Therefore, an optimization control strategy of GAs was developed to acquire optimal temperature profiles.

Optimization of the temperature for non-isothermal SSF process was made using the optimal profile. The prediction of the stated variables such as maltotriose, maltose and ethanol are shown as different type

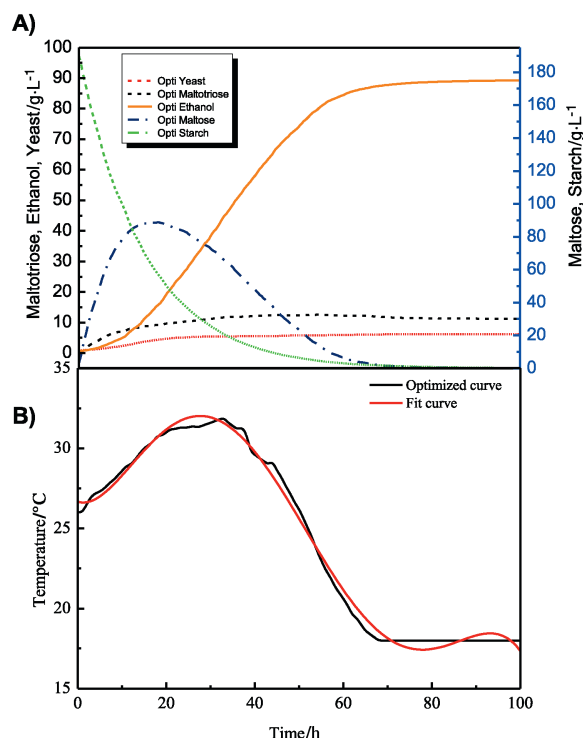


Fig. 5. Optimized temperature profile and ethanol production by SSF during Chinese wine fermentation.

lines in Fig. 5A. The model predicted the temperature profile was nonlinear (Fig. 5B).

The temperature profile estimated by Origin 8.0 can be described by the equation shown below:

$$T = 26.66642 - 0.08933 \times t + 0.038 \times t^2 - 0.00144 \times t^3 + 1.76311 \times 10^{-5} \times t^4 - 7.05567 \times 10^{-8} \times t^5 \quad (13)$$

where, t is time. Temperature profiling begins with the low temperature at 26 °C, which allows the growth of the yeast cells during the first 20 h. Subsequently, the operating temperature increased rapidly up to 31.97 °C at 30 h, after which it decreased slowly to 18 °C at 65 h. The predicted final concentration of ethanol was 89.3 g·L⁻¹, which is 14%–21% higher than that of the traditional SSF process (manual controlling).

4. Conclusions

The effect of constant temperature on Chinese rice wine fermentation and optimal temperature profile was investigated at a scale-down level using Mathematical model was developed ematical model. The operating temperature of Chinese rice wine fermentation was restricted by the temperature for the reason that high temperature will cause excessive organic acid production. Thus, the upper and lower limits of operating temperature are 18 and 33 °C, respectively, in this work. The accordingly developed temperature-dependent model has good agreement with the experimental data. Our model suggests that temperature has a strong effect on starch hydrolysis as well as cell growth and ethanol production. The optimal temperature profile is: during the first 20 h the temperature increases gradually from 26 °C to 31.9 °C. The operating temperature at around 31.5 °C lasted for 13 h. Thereafter, it decreases slowly to 18 °C at 68 h. The predicted final concentration of ethanol is 89.3 g·L⁻¹. Our model conceivably can facilitate the improvement of the production of Chinese rice wine at the industrial scale.

Nomenclature

A	ethanol concentration, g·L ⁻¹
C	cell mass concentration, g·L ⁻¹
C_1	starch-qu complex concentration, g·L ⁻¹
E	Qu concentration, g·L ⁻¹
$K_{s1}-K_{s6}$	saturation constant, g·L ⁻¹
k_1-k_6	chemical reaction rates, g·L ⁻¹ ·h ⁻¹
M	maltose concentration, g·L ⁻¹
O	oxygen concentration, g·L ⁻¹
R	maltotriose concentration, g·L ⁻¹
S	starch concentration, g·L ⁻¹

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