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Research Article

Fermentation Process Modeling with Levenberg-Marquardt Algorithm and Runge-Kutta Method on Ethanol Production by *Saccharomyces cerevisiae*

Dengfeng Liu,[1] Ling Xu,[1,2] Weili Xiong,[1] Hong-Tao Zhang,[3] Chi-Chung Lin,[3] Lihua Jiang,[1] and Baoguo Xu[1]

[1] *Key Laboratory of Industrial Advanced Process Control for Light Industry, Ministry of Education, Jiangnan University, Wuxi 214122, China*
[2] *School of Internet of Things Technology, Wuxi Institute of Commerce, Wuxi 214153, China*
[3] *Key Laboratory of Industrial Biotechnology, Ministry of Education, Jiangnan University, Wuxi 214122, China*

Correspondence should be addressed to Baoguo Xu; xbg@jiangnan.edu.cn

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The core of the Chinese rice wine making is a typical simultaneous saccharification and fermentation (SSF) process. In order to control and optimize the SSF process of Chinese rice wine brewing, it is necessary to construct kinetic model and study the influence of temperature on the Chinese rice wine brewing process. An unstructured kinetic model containing 12 kinetics parameters was developed and used to describe the changing of kinetic parameters in Chinese rice wine fermentation at 22, 26, and 30°C. The effects of substrate and product inhibitions were included in the model, and four variable, including biomass, ethanol, sugar and substrate were considered. The *R*-square values for the model are all above 0.95 revealing that the model prediction values could match experimental data very well. Our model conceivably contributes significantly to the improvement of the industrial process for the production of Chinese rice wine.

1. Introduction

Chinese rice wine is an important alcoholic beverage made from whole steamed sticky rice (also known as glutinous rice) in China with a long history. At this time, the production of Chinese rice wine has reached 1.4 million tons per year. Thus far, traditionally the rice wine fermentation process is still manually controlled by technicians based on their experience. This caused variability of the flavour for each batch of Chinese rice wine. Hitherto, how to maintain and standardize the flavour of all batches of rice wine is still an unsolved issue. To achieve optimal automatic bioreactor control in the rice wine fermentation production process is expected to resolve such problem. Development of a robust mathematical model on the optimal automatic control fermentation system for Chinese rice wine production is absolutely necessary [1–3]. Whole sticky rice and Chinese wheat *qu* are the two key raw materials used for Chinese rice wine brewing. Chinese wheat *qu* is a starter culture containing predominately the fungus *Aspergillus oryzae* and enzymes as saccharifying and fermenting agents. To make Chinese wheat *qu*, the wheat grain is milled, mixed with natural water, and pressed into a starter cake. It is subsequently incubated at 28–30°C for 48 h and dried at 45°C until the moisture is lower than 12% (w/w) [4, 5]. The Chinese rice wine brewing process can be divided into two stages as follows: whole rice was soaked first and then steamed. Finally, the steamed sticky rice, Chinese wheat *qu*, and tap water were mixed at an appropriate ratio and naturally fermented at lower than 33°C condition for 96 h (the main stage). The resulting broth was pumped into a fermentor tank at 15°C for another 45 days (the second stage). In all the processes of Chinese rice wine brewing, the first stage is the key step for acquiring high Chinese wine quality and yield. Consequently, we focused on developing an unstructured model to describe the main stage of the fermentation process.

The main-stage fermentation process of rice wine production is typically simultaneous saccharification and fermentation (SSF) process. It is divided into two stages: the first stage is enzymatic saccharification and microbial growth, and the second stage is enzymatic saccharification and microbial fermentation (ethanol production). During the enzymatic saccharification process the polysaccharides from whole steamed sticky rice are hydrolyzed into reducing sugar and oligosaccharides for the fermentation process. The reducing sugar is converted into ethanol and flavour agent through the Chinese rice wine fermentation production process.

There are several kinetic models in industrial alcoholic beverage fermentation to describe the SSF process of ethanol production with different materials such as corn flour [6, 7], wheat flour [8, 9], and some cellulosic substrates [10–15]. Hitherto, these SSF models are mainly classified as two types: unstructured model and cybernetic model. In cybernetic modeling, the core parts are the description of the synthesis rates of key enzymes and the metabolic flux balance of the equation of the enzymes in the Chinese wheat *qu*. As the saccharification process of Chinese rice wine was conducted with Chinese wheat *qu* containing enzymes, cybernetic modeling cannot explain this situation. Kroumov et al. [16] developed an unstructured model for simultaneous saccharification and fermentation of starch to ethanol with the recombinant strain of *S. cerevisiae* YPB-G. In this case, *S. cerevisiae* YPB-G can produce both saccharification enzymes and ethanol. Therefore, new saccharification enzymes were induced in the fermentation process. It is different from that of Chinese rice wine brewing during which saccharification enzymes are not induced endogenously. Chavan et al. [17] modeled the SSF process from starch to flavour compounds. Podkaminer et al. [11] found ethanol concentration can partly inactivate the enzyme and then developed a kinetic model to model this phenomenon for thermophilic SSF at 50°C with *Thermoanaero saccharolyticum* ALK2. These SSF kinetic models provided useful information for developing the kinetic models to describe the SSF process in Chinese rice wine fermentation.

There are many kinetic models to describe the SSF processes. However, all existing models cannot apply to the conditions of Chinese rice wine brewing. Chinese wheat *qu* and sticky rice are the main raw materials for producing reducing sugar. The Chinese wheat *qu* mainly contains amyloglucosidase and α-amylase which hydrolyze the starch in the sticky rice to reducing sugar [5]. *Saccharomyces cerevisiae* yeast was used to convert the reducing sugar into ethanol [18]. In the Chinese rice wine fermentation production, there are several factors which are different from other SSF processes: (1) whole steamed rice instead of starch flour is used as source to supply starch which is then hydrolyzed to reducing sugar; (2) in the Chinese rice wine brewing process, Chinese wheat *qu* which contains α-amylase and amyloglucosidase is used to complete the saccharification of rice; (3) variable temperatures are used up to 33°C. (4) The concentration of sugars can reach as high as 150 g L^{-1}; (5) final ethanol concentration in Chinese rice wine brewing can reach as high as 21% (v/v). The brewing process condition for Chinese rice wine is different from the alcohol fermentation process. Therefore, we cannot directly apply the alcohol fermentation kinetic models to describe the Chinese rice wine fermentation process. It is necessary to develop a new and specific SSF model to describe the Chinese rice wine brewing process. In addition, although temperature is the key factor affecting the Chinese rice wine, the Chinese wheat *qu* quality and yield are also very important. Hitherto, there are only limited publications on the study of the effect of temperature for Chinese rice wine fermentation production.

Consequently, we focused on (1) analyzing of the effect of various temperatures on Chinese rice wine fermentation, (2) formulating the kinetic model on reducing sugar and ethanol production during Chinese rice wine fermentation, (3) validating the model with experimental data of the SSF process in Chinese rice wine fermentation using the Chinese wheat *qu*, sticky rice, *Saccharomyces cerevisiae* to develop model parameters from our experimental data at various temperatures using the least-squares algorithm.

2. Material and Methods

2.1. Microorganisms for Fermentation. The yeast, *Saccharomyces cerevisiae* EC1118 (Lallemand Australia Pty Ltd, Underadale, SA, Australia), was used in this study and stored at 4°C. The Chinese wheat *qu* used in this study was supplied by the Shaoxing Nu′er Hong Rice Wine Company (Zhejiang, China) and stored at room temperature. Sticky (glutinous) rice purchased from a local store in Columbia, MO (product of Thailand, distributed by Walong Marketing Inc., Buena Park, CA, USA) was used in this study.

2.2. Fermentation and Sample Preparation. To analyze the saccharification process, steamed rice, Chinese wheat qu and water at the ratio of 10 : 2 : 30 was prepared under three different temperatures (22, 26, and 30∘C) [18]. At the predetermined time, samples of 5 mL cultures were taken from the fermentor and for analyses. Reducing sugar and pH were recorded with pH meter (S220 SevenCompact pH/Ion) and UV spectrometer (Thermo Scientific Spectronic Gensys 5 UV Spectrometer) at 600 nm.

The Chinese rice wine fermentation experiments were carried out in 1000 mL shake flask (600 mL working volume) at 22, 26, and 30°C without pH control. Samples of 5 mL of the fermentation broth were taken for analyses of reducing sugar [19] and pH. 20 mL of broth was taken for analyzing the ethanol concentration. The Chinese rice wine fermentation was conducted with the medium containing steamed rice, Chinese wheat qu and water at the ratio of 10 : 2 : 30. *Saccharomyces cerevisiae* EC118 was added to water and heated to 40°C for 20 min at the ratio of 500 g : 5 L water. Subsequently, 20 mL of the resulting broth was added to each flask.

As the Chinese rice wine fermentation is at half-solid state and half-liquid process and the sticky rice is always in the fermentation broth, it is difficult to determine the biomass of *Saccharomyces cerevisiae* for which 20 mL of *Saccharomyces cerevisiae* EC118 was added to the fermentation medium under three different temperatures (22, 26, and 30°C) for 96 h. All the cultures were taken out at various times (see Section 3)

and were analyzed in the following section. This medium only contains glucose with concentration at 150 g L^{-1}.

2.3. Analytical Methods

2.3.1. Determination of Biomass. For biomass determination, 5 mL of fermentation mash was taken out and centrifuged at 6,000 g for 4 min. The cells were suspended in 5 mL distilled water. The optical density was determined at 600 nm with the UV spectrometer. If the OD_{600} is above 1, the sample was diluted and then tested until the DO_{600} is less than 1. Dry cell weight (X) was determined with cell pellet dried at 80°C in an oven for 48 h. The standard curve of cell biomass was obtained as below based on the linear relation of OD value (y) and dry cell weight (X):

$$X = 0.668y - 0.0062, \quad \left(R^2 = 0.996\right), \tag{1}$$

where X is dry cell weight and g L^{-1}; OD_{600} is optical density at 600 nm.

2.3.2. Determination of the Quantity of Reducing Sugar in the Chinese Wine Fermentation Broth. For the determination of the reducing sugar (G) concentration, the fermentation broth was taken and centrifuged, and then 20 μL was diluted to 2 mL with distilled water and mixed with 1.5 mL 3,5-dinitrosalic acid reagent [19]. After it was heated to 100°C for 10 min, samples were diluted to 25 mL and then determined with UV spectrometer at 520 nm. The standard curve of reducing sugar was obtained as described below based on the linear relation of OD_{520} value (z) and reducing sugar (G):

$$G = 0.7294z + 0.0344, \quad \left(R^2 = 0.999\right), \tag{2}$$

where G is reducing sugar concentration, g L^{-1}; OD_{520} is optical density at 520 nm from UV spectrometer.

2.3.3. Determination of the Concentration of Ethanol. An ebulliometer (Napa, CA, USA) was used to determine the alcohol content in the Chinese rice wine (http://www.dujardin-salleron.com/societe/index.php?langue=2). The test is based on the difference between the boiling points of water and wine. The procedure is as follows: (1) determine the boiling point of water; (2) dilute the wine sample so that the boiling point of the diluted wine is within 4°C of the boiling point of water; (3) calculate the concentration of the wine with the standards value indicated by the instrument.

2.4. Curve Fitting and Parameter Identification Procedure. Based on model, there are 12 parameters which need to be estimated with data from the experiments under various temperatures in this work. They are $k_1, k_2, k_3, k_4, Y_{x/s}, Y_{p/s}, k_0, k_s, k_{s1}, k_{ps1}, k_{pi1}, k_m$. They can be expressed with the following equation:

$$C = f\left(k_1, k_2, k_3, k_4, Y_{x/s}, Y_{p/s}, k_0, k_s, k_{s1}, k_{ps1}, k_{pi1}, k_m\right). \tag{3}$$

To obtain an ideal kinetic model, the optimization and reoptimization steps of the identification procedures were performed using the Levenberg-Marquardt method and the fourth-order Runge-Kutta method. The objective function (e) was used:

$$e = \sum_i \frac{\left(X_i - \widehat{X}_i\right)^2}{\widehat{X}_i^2} + \sum_i \frac{\left(E_i - \widehat{E}_i\right)^2}{\widehat{E}_i^2} + \sum_i \frac{\left(G_i - \widehat{G}_i\right)^2}{\widehat{G}_i^2}, \tag{4}$$

where, X_i, E_i, G_i are the experimental values of the dry cell weight biomass, ethanol, and reducing sugar, respectively. On the contrary, $\widehat{X}_i$, $\widehat{E}_i$, and $\widehat{G}_i$ are the values predicted by the model. Parameters estimate and identification of the model were realized through minimizing e value of (4). The nonlinear regression analysis in accordance with the Levenberg-Marquardt method [20] was used to minimize the objective function (e), which has been successfully used in the optimization parameters in the other kinetic models [21–24]. The model was solved by using the fourth-order Runge-Kutta method ode 45 with the MATLAB R2009a software. Both the fourth-order Runge-Kutta method and the Levenberg-Marquardt method were also applied to optimize the estimated parameters with the MATLAB R2009a software [20].

3. Results and Discussion

During the Chinese rice wine fermentation, temperature plays important role in the reducing sugar, ethanol production, and its flavour formation. Consequently, it is necessary to study the kinetic of yeast growth and production of *Saccharomyces cerevisiae* under various temperatures. The effect of temperature on ethanol production has been studied above 30°C [25, 26]. However, as the temperature in the Chinese rice wine fermentation process is lower than 31°C, it is necessary to explore the effect of temperature under 30°C.

3.1. Cell Growth at Various Temperatures. *Saccharomyces cerevisiae* cell growth showed a classical cell growth trend under various temperatures (Figure 1). The batch fermentation process of Chinese rice wine can be separated into different stages. The first stage is the lag phase. In this stage, the biomass increased slowly, and then the biomass increased faster in the subsequent exponential growth phase. Finally, 5.2 g L^{-1} of biomass was attained at 26°C in the stationary phase. The highest cell biomass was achieved at 26°C which is most suitable for yeast growth. Reducing sugar formation began to form as soon as the fermentation started. However, ethanol production was initiated when the yeast cells reached the exponential phase and cell growth occurred simultaneously. Similar phenomenon has been reported [25].

3.2. Reducing Sugar Formation and Consumption at Various Temperatures. To evaluate the reducing sugar production at various temperatures, fermentation process only with the Chinese wheat *qu* treatment was performed. Figure 2(a) shows that reducing sugar increased with the fermentation time. However, the improvement of reducing sugar formation at 30°C was higher than that at other temperatures

as well as from 2 h to 25 h. Reducing sugar formation declined until 60 h. Thereafter, the reducing sugar concentration was sharply lower. It suggested the *Aspergillus oryzae* fungus in the Chinese wheat *qu* also consumed the reducing sugar but did not affect the *Saccharomyces cerevisiae* yeast, which used the reducing sugar to produce ethanol.

Figure 2(b) shows the reducing sugar profile under various temperatures. A maximum reducing sugar concentration of 140 g L^{-1} was accumulated in the fermentation broth at 22 h under 30°C. The profile of the reducing sugar indicates that during the initial stage the saccharification rate was higher than the fermentation rate leading to the accumulation of the reducing sugar. The yeast was in the lag phase with lower consumption of reducing sugar for cell growth. Subsequently, the yeast entered the exponential stage during which reducing sugar was consumed. The maximum reducing sugar concentrations in the broth were 130 g L^{-1}, 130 g L^{-1}, and 140 g L^{-1} at 22, 26, and 30°C, respectively. The reducing sugar concentration reached the peak at 30°C higher than that at 26°C. The reducing sugar concentration that reached peak level at 22°C nearly 10 h later than that at 26 or 30°C. These results suggested that at the saccharification stage (0 to 50 h) higher temperature facilitated reducing sugar formation.

High temperature can provide positive effect on the cell biomass growth and ethanol production at first 50 h. After 50 h, low temperature is better for cell maintenance and enzymatic activity which enhance ethanol production. Therefore, ideal rice wine fermentation can be improved by a two-stage temperature control strategy to increase the ethanol production level and reduce organic acid production.

3.3. Ethanol Production and pH at Various Temperatures. Ethanol production by *Saccharomyces cerevisiae* showed a typical trend at various temperature levels (Figure 3(a)). The highest cell biomass was achieved at 26°C. However, highest ethanol production was at 22°C. The levels of ethanol and cell biomass were compared from 0 to 40 h. The cell biomass at 30°C attained the highest level of 6 g L^{-1}. The ethanol production at 30°C at first 50 h is at the highest compared with other conditions. However, the ethanol production at 22°C increased quickly after 50 h and the ethanol concentration accumulation at 22°C reached the highest value of 11% (v/v). These results suggest that both ethanol production and cell growth were affected by temperature. During the first 50 h, higher temperature was suitable for cell growth and ethanol production. However after 50 h, the lower temperature was better for ethanol production. Temperature, which can affect the production of ethanol, was also observed by other related researches [27, 28].

For pH values, similar changing profiles were observed at different temperature (Figure 3(b)) which shows that pH decreased dramatically during the first 40 h under 26 and 30°C conditions. It suggests that high temperatures will enhance the organic acid production and will cause pH to drop more quickly than that at low temperatures.

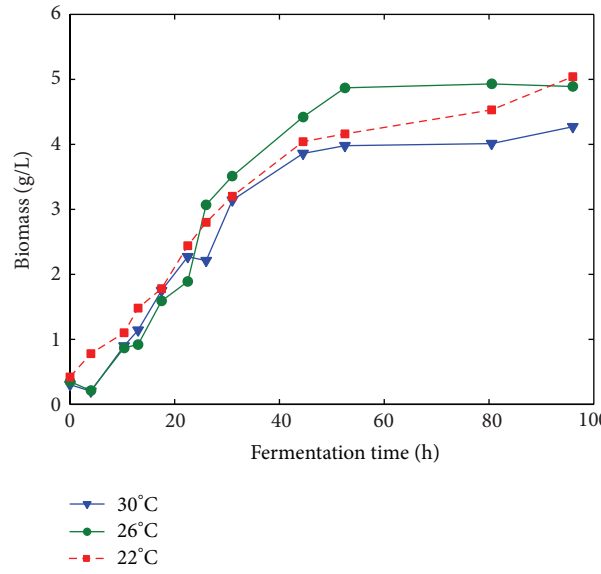

Figure 1: Kinetic model of SSF process in rice wine fermentation.

3.4. Model Development. The system considered by the unstructured model is composed of four main variables: starch (S), reducing sugar (G), dry cell weight (X), and ethanol concentrations (E). The chemical reactions for this process are shown in Figure 4.

3.4.1. Saccharification of Starch to Reducing Sugar. During Chinese rice wine fermentation, the reducing sugar from the enzymatic hydrolyzed starch was used to produce ethanol and/or maintain cell growth. The net reducing sugar accumulation rate is

$$\frac{dG}{dt} = r_f - r_u, \tag{5}$$

where r_f is reducing sugar formation and r_u is reducing sugar consumption used for fermentation with *Saccharomyces cerevisiae*.

The Michelis-Menten kinetics, including competitive inhibition of reducing sugar, was used to describe the enzymatic saccharification of starch to reducing sugar in Chinese rice wine fermentation process. Thus,

$$r_s = k_1 \frac{S}{k_m\left(1 + \left(G/k_0\right)\right) + S}. \tag{6}$$

The assumed model in the saccharification level is based on the model to describe starch saccharification [16]. In this model, the *Saccharomyces cerevisiae* EC118 was used for the fermentation, and Chinese wheat *qu* was used for the saccharification.

Reducing sugar was used to evaluate the sugar concentration in fermentation broth of rice wine fermentation process. In this work, the reducing sugar was considered as glucose.

As for the exact ratio of α-amylase and amyloglucosidase in the Chinese wheat *qu*, α-amylase and amyloglucosidase

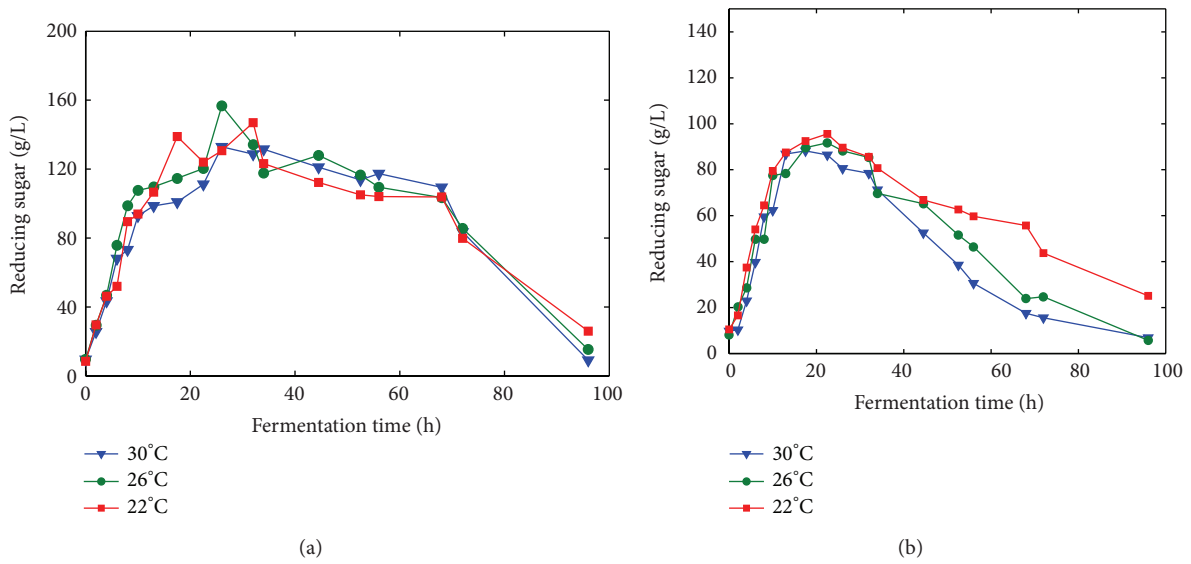

FIGURE 2: Experimental residue reducing sugar curve of the batch fermentation at various temperatures; (a) only added Chinese wheat *qu* and (b) added Chinese wheat *qu* and *S. cerevisiae*.

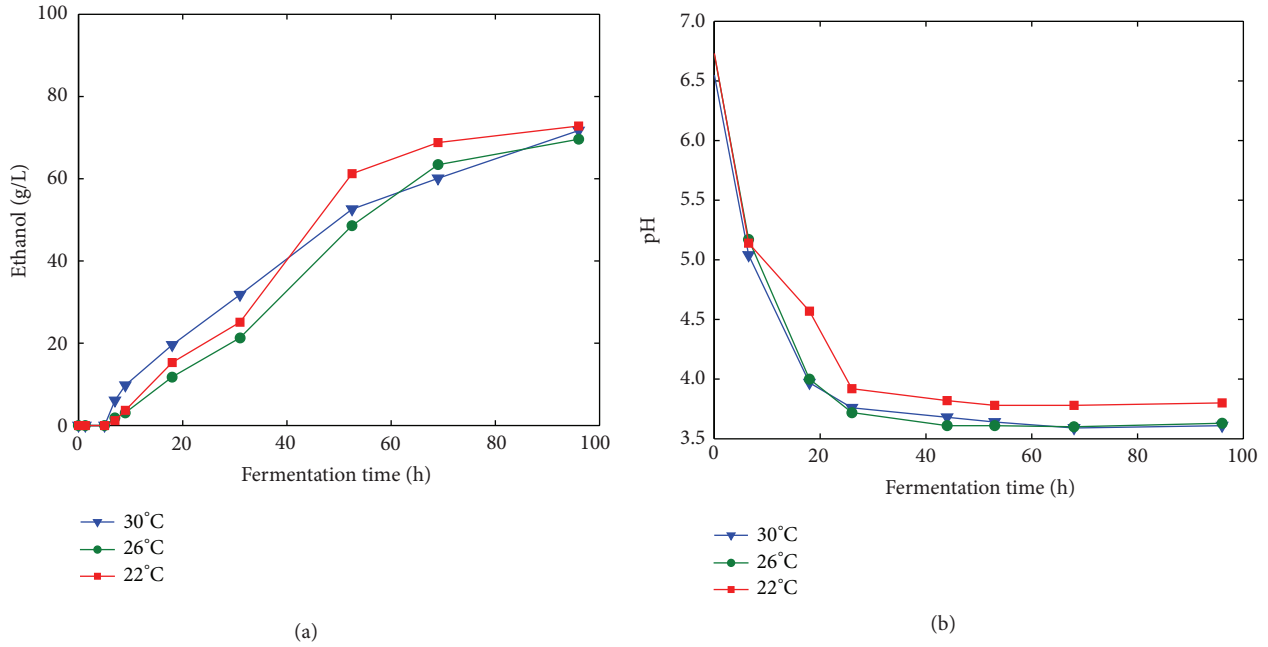

FIGURE 3: Experimental residue reducing sugar curve of the batch fermentation at various temperatures; (a) only added Chinese wheat *qu* and (b) added Chinese wheat *qu* and *S. cerevisiae*.

were considered as a "complex enzyme." For simplification of the model, the concentration of enzyme was considered as constant. The enzymatic affinity to the starch is independent of enzyme concentration.

The mass transfer limitations and conformational changes of the enzyme structure were not considered in the model.

Ethanol was considered as the main products; other flavour compounds were not considered in this work.

3.4.2. Microbial Growth. Part of the sugar was consumed by the cells for growth. The specific growth rate of yeast was described as the Monod equation with substrate inhibition below:

$$\mu = k_2 \frac{G}{k_s + G}. \tag{7}$$

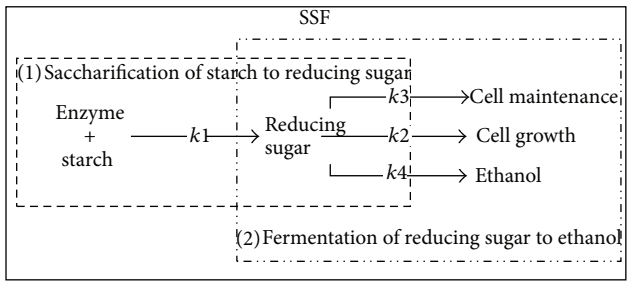

Figure 4: Kinetic model of SSF process in rice wine fermentation.

The growth rate of yeast cell was assumed to be directly proportional to the dry cell weight; so the growth rate of cells is written as

$$\frac{dX}{dt} = \mu X, \tag{8}$$

where X is the dry cell weight and G is reducing sugar concentration. K_s is the inhibition coefficient.

Some of the sugar was consumed by cells for cellular maintenance. The sugar needed was directly proportional to the dry cell weight. It is described in

$$r_m = k_3 X. \tag{9}$$

3.4.3. Fermentation of Reducing Sugar to Ethanol. Ethanol was the metabolic product of *Saccharomyces cerevisiae* fermentation. Ethanol is assumed to be directly proportional to the dry cell weight and substrate as well as reducing sugar concentration. The effects of the reducing sugar and metabolic product ethanol inhibitions were considered in the fermentation process from reducing sugar to ethanol. The formation rate of ethanol from reducing sugar is written as follows:

$$r_E = k_4 \frac{1}{(k_{s1} + G)} \frac{1}{\left(k_{\text{ps1}} + E + \left(E^2/k_{\text{pi1}}\right)\right)} GX, \tag{10}$$

where $1/(k_{s1} + G)$ is the substrate inhibition and $1/(k_{\text{ps1}} + E + (E^2/k_{\text{pi1}}))$ is the product inhibition.

3.4.4. Model of the SSF Process. The model of SSF process is described as follows based on (5)–(10).

Starch balance:

$$\frac{dS}{dt} = -k_1 \frac{S}{k_m\left(1 + \left(G/k_0\right)\right) + S}. \tag{11}$$

Biomass balance:

$$\frac{dX}{dt} = k_2 \frac{G}{k_s + G} X. \tag{12}$$

Ethanol balance:

$$\frac{dE}{dt} = r_E. \tag{13}$$

Reducing sugar balance:

$$\frac{dG}{dt} = r_f - r_u, \tag{14}$$

where

$$r_f = 1.11 k_1 \frac{S}{k_m\left(1 + \left(G/k_0\right)\right) + S}, \tag{15}$$

$$r_u = \frac{1}{Y_{x/s}} \frac{dX}{dt} + r_m + \frac{1}{Y_{p/s}} \frac{dE}{dt}. \tag{16}$$

3.4.5. Solving Model. Since the kinematic model of the SSF in rice wine fermentation is nonlinear, therefore, only numerical solution is available but not analytical solution. In this work, the Runge-Kutta method was used to get the numerical solution for the model. The initial condition was set as x_0, and x_i was achieved as numerical solution by recursive relation equation:

$$\begin{aligned}
x_{i+1,j} &= x_{ij} + \frac{1}{6}\left(b_{1j} + 2b_{2j} + 2b_{3j} + b_{4j}\right) + O\left(h^5\right), \\
b_{1j} &= hf_j\left(t_i, x_{i1}, x_{i2}, \ldots, x_{in}\right), \\
b_{2j} &= hf_j\left(t_i + \frac{h}{2}, x_{i1} + \frac{b_{11}}{2}, x_{i2} + \frac{b_{12}}{2}, \ldots, x_{in} + \frac{b_{1n}}{2}\right), \\
b_{3j} &= hf_j\left(t_i + \frac{h}{2}, x_{i1} + \frac{b_{21}}{2}, x_{i2} + \frac{b_{22}}{2}, \ldots, x_{in} + \frac{b_{2n}}{2}\right), \\
b_{4j} &= hf_j\left(t_i + h, x_{i1} + b_{31}, x_{i1} + b_{32}, \ldots, x_{in} + b_{3n}\right).
\end{aligned} \tag{13'}$$

3.5. Validating and Estimating Parameters of Unstructured Model for Chinese Rice Wine Fermentation. Several models and parameters related to only starch hydrolysis have been constructed and evaluated with α-amylase [29] and amyloglucosidase [30]. However, no study on the effect of various temperatures on ethanol fermentation was undertaken with their models. Therefore, it is interesting to analyze the effect of a series of temperatures on ethanol production. To acquire kinetic parameters of the kinetic model for Chinese rice wine fermentation under various temperatures, the simulated Chinese rice wine fermentation process at different temperatures in a scale-down level was done, considering the temperature in Chinese rice wine brewing was controlled below 33°C. Accordingly, 22, 26, and 30°C were chosen and controlled for the simulated Chinese rice wine fermentation. The data and simulation are shown in Figures 5(a), 5(b), and 5(d). The data are presented in data points and simulation is presented with solid lines for reducing sugar (G), biomass (X), and ethanol (E), respectively.

All the optimized parameters were calculated and fitted the modeling data with the experimental data at 22, 26, and 30°C. The R-square value was used to evaluate the model fitting. The R^2 value more close to 1, the model fitting is considered to be better. The R-square values of the model under different temperatures which are calculated by $R^2 = 1-$

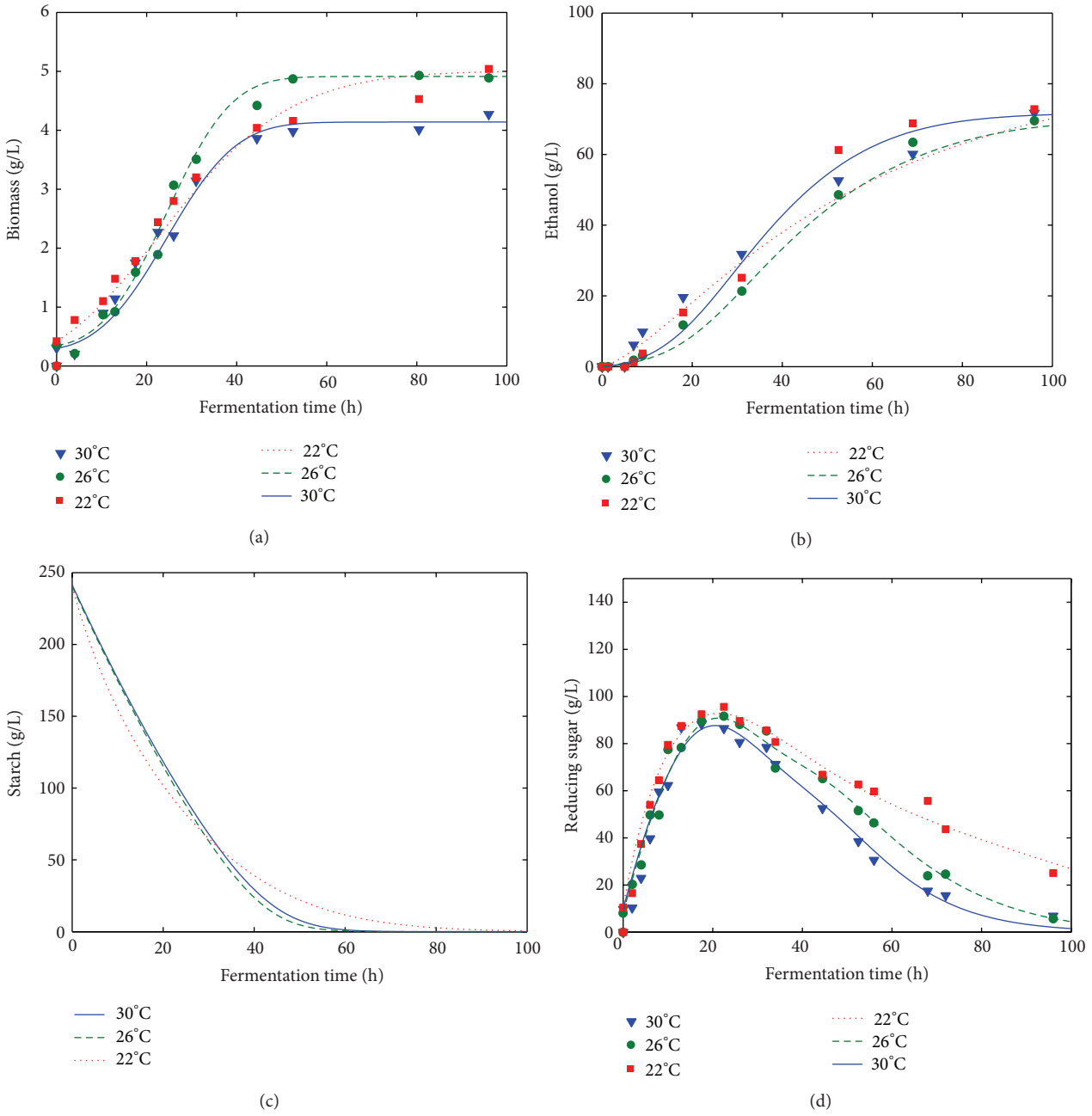

FIGURE 5: Experimental data and simulated data for batch experiments at 22, 26, and 30°C (a). Cell mass concentration. (b) Ethanol concentration. (c) Starch concentration. (d) Reducing sugar concentration.

TABLE 1: R-square value for the model.

State varible	Temperature		
	22°C	26°C	30°C
Reducing Sugar	0.96	0.97	0.95
Cell mass	0.98	0.99	0.98
Ethanol	0.99	0.99	0.99

$(\sum(x_i - \hat{x}_i)^2 / \sum(x_i - \bar{x}_i)^2)$ are shown as Table 1. The R-square values of reducing sugar are 0.96 at 22°C, 0.97 at 26°C, and 0.95 at 30°C, respectively. The R-square values of cell mass under the temperatures from 22 to 30°C are 0.99, 0.98, and 0.99, respectively. The R-square values of ethanol are all 0.99 at all three different temperatures. The plot figures (Figure 5) and R-square values show that the model fit the data very well. The model thus is very useful to describe the Chinese rice wine fermentation process.

The kinetic parameters at all temperatures are shown in Table 2. The maximum rate of saccharification (k_1) was increased from 6.772 g L^{-1} h^{-1} at 22°C to 7.721 g L^{-1} h^{-1} at 30°C with the temperature increasing. Maximum specific

Table 2: Kinetic and stoichiometric parameters values and initial conditions of the model.

Evaluated parameter	Temperature			
	22°C	26°C	30°C	Units
k_1	6.772	7.099	7.721	$g\,L^{-1}\,h^{-1}$
k_2	0.110	0.120	0.127	$g\,L^{-1}\,h^{-1}$
k_3	0.001	0.001	0.001	h^{-1}
k_4	99.399	105.383	135.393	$g\,L^{-1}\,h^{-1}$
k_m	31.856	31.809	32.436	$g\,L^{-1}$
k_0	139.959	139.964	139.887	$g\,L^{-1}\,h^{-1}$
$Y_{x/s}$	0.894	0.882	0.651	$g\,g^{-1}$
k_s	116.533	104.533	104.596	$g\,L^{-1}$
$Y_{p/s}$	0.254	0.244	0.257	$g\,g^{-1}$
k_{s1}	60.984	60.984	61.344	$g\,L^{-1}$
k_{ps1}	56.256	56.287	56.176	$g\,L^{-1}$
k_{pi1}	170.778	170.779	170.756	$g\,L^{-1}$

growth rate (k_2) was increased from 0.110 at 22°C to 0.127 at 30°C. Consumption coefficient for cell maintenance (k_3) contains the same value $0.001\,h^{-1}$ under various temperatures. The maximum ethanol production rate (k_4) increased from $99.399\,g\,L^{-1}\,h^{-1}$ at 22°C to 135.393 at 30°C also. Glucose inhibition constant for saccharification (k_0), saturation growth constant (k_{s1}), substrate growth inhibition constant (k_{ps1}), substrate production inhibition term (k_{pi1}), and yield coefficient of product ($Y_{p/s}$) all only have little fluctuation under various temperatures. However, the yield coefficient of cell growth ($Y_{x/s}$) was decreased from $0.894\,g\,g^{-1}$ at 22°C to $0.651\,g\,g^{-1}$ at 30°C.

The model of biomass is quite accurate and there was no drastic mismatch between the experimental and simulation data (Figure 5(a)). The effect of intermediate products of reducing sugar from starch hydrolysis on cell growth and ethanol production can be very significant. The Chinese rice wine fermentation is a typical SSF process in which at the initial stage the reducing sugar concentration is low but the starch concentration is higher. In this model, total reducing sugar was used as sugar contents. The computation model for reducing sugar concentration and ethanol production fits very well to the profile of experimental data (Figures 5(b) and 5(d)). It is to be noted that the computation model for ethanol production cannot fit very well at 22°C from 0 to 60 h (Figure 5(b)). The utilization of glucose at 22°C is lower than the other conditions, but the glucose formation is similar to the other conditions and ethanol concentration reached the highest value. The pH values at low temperature were higher than that at high temperature (Figure 3(b)) because organic acid produced more at higher temperature in the fermentation broth. All the results suggested that high temperature enhanced glucose to produce organic acid but not ethanol.

In addition, high temperature can increase the values of maximum rate of saccharification, maximum specific growth rate, and maximum ethanol production rate. However, yield coefficient for cell growth and saturation growth constant are decreased with the temperature increasing. It is to be noted that the parameters values of yield coefficient of product, saturation growth constant, substrate growth inhibition constant, and substrate production inhibition term cannot be affected apparently by the temperature.

Overall, exploring the fermentation process provides very useful information for industrial ethanol fermentation to reduce power consumption and increases production. At present, kinetic modeling is an indispensable step in exploring and developing a better fermentation process since the models can be used to determine an optimal operational condition for the production of a target metabolite. However, an unstructured kinetic model to study the temperature on ethanol production in Chinese rice wine fermentation is still not available until now. Therefore, our new model suitable for rice wine fermentation and analysis of the effect of temperatures on ethanol production contributes significantly to the improvement of the industrial production process for Chinese rice wine.

4. Conclusion

SSF is an essential industrial technology for converting bioresources into useful products, such as lactic acid and ethanol [31–35]. As ethanol can be used as biofuel, producing ethanol from bioresource is becoming very important bioprocess to resolve the energy crisis [36, 37], and several models have been developed to describe the SSF process from wood, corn flower, and starch [38, 39]. The SSF process uses whole rice and Chinese wheat *qu*, which contains mostly α-amylase and amyloglucosidase, to produce reducing sugar and ethanol with *Saccharomyces cerevisiae*. Additionally, ethanol production achieved as high as 21% (v/v), compared to only 11% concentration in industrial ethanol fermentation. Furthermore, the temperature is limited to below 31°C [18, 40] in this process. Consequently, exploring the fermentation process provides very useful information for industrial ethanol

fermentation to reduce power consumption and increases production. At present, kinetic modeling is an indispensable step in exploring and developing a better fermentation process since the models can be used to determine an optimal operational condition for the production of a target metabolite. However, an unstructured kinetic model to study the temperature on ethanol production in Chinese rice wine fermentation is still not available until now. Therefore, our new model suitable for rice wine fermentation and analysis of the effect of temperatures on ethanol production contributes significantly to the improvement of the industrial production process for Chinese rice wine.

Nomenclature

S: Starch concentration ($g\,L^{-1}$)
G: Reducing sugar concentration ($g\,L^{-1}$)
X: Biomass concentration ($g\,L^{-1}$)
E: Ethanol concentration ($g\,L^{-1}$)
μ: Specific growth rate of yeast (h^{-1})
r_m: Consumption rate for cell maintenance ($g\,L^{-1}\,h^{-1}$)
r_E: Formation rate of ethanol from reducing sugar ($g\,L^{-1}\,h^{-1}$)
r_f: Reducing sugar formation rate ($g\,L^{-1}\,h^{-1}$)
r_u: Reducing sugar utilization rate ($g\,L^{-1}\,h^{-1}$)
k_1: Maximum rate of saccharification ($g\,L^{-1}\,h^{-1}$)
k_2: Maximum specific growth rate ($g\,L^{-1}\,h^{-1}$)
k_3: Consumption coefficient for cell maintenance (per h)
k_4: Maximum of ethanol production rate ($g\,L^{-1}\,h^{-1}$)
$Y_{p/s}$: Yield coefficient of product ($g\,g^{-1}$)
$Y_{x/s}$: Yield coefficient of cell growth ($g\,g^{-1}$)
k_0: Glucose inhibition constant for saccharification ($g\,L^{-1}$)
k_m: Inhibition constant for saccharification ($g\,L^{-1}$)
k_s: Saturation growth constant ($g\,L^{-1}$)
k_E: Ethanol inhibition constant ($g\,L^{-1}$)
k_{s1}: Saturation growth constant ($g\,L^{-1}$)
k_{ps1}: Substrate growth inhibition constant ($g\,L^{-1}$)
k_{pi1}: Substrate production inhibition term ($g\,L^{-1}$).

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

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