Fermentation Kinetics of Potato Liquor

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Abstract: *Potato liquor is mainly produced through cooking, fermenting and aging of potatoes, plus some corn, sorghum and duck wheat. In this paper, activated dry yeast is adopted for constant temperature fermentation, and kinetic models are set up based on logistic function for the yeast growth, alcohol formation and substrate consumption in the fermentation process. The number of yeast cells, alcohol content and sugar content of the fermentation broth were measured every other day. The kinetic features of each test parameter were analyzed through observation and results analysis. The research provides insights into the physiological features of relevant microorganisms, the growth rate of yeast, the optimal conditions for alcohol formation, and the relationship between related parameters. These results lay a solid basis for the control of fermentation technology.*

Keywords: *Potato, Liquor, Fermentation, Kinetic Model.*

Introduction

Potato liquor is an alcoholic beverage produced mainly from potato, plus some corn, sorghum and duck wheat. The production processes include cooking, fermenting, aging and filtering. The Liangshan region is a leading producing area of potato in China. This region owns wider potato fields and harvests more potatoes than anywhere else in Sichuan Province. The R&D of new potation products (e.g., new wine) helps to fully utilize the local resources and promote the sales of Liangshan potatoes.

The fermentation kinetics mainly deals with the variation in microbial growth, formation of alcohol and the total sugar consumption of the substrate. It provides an effective way to analyze the test process and predict various test indices. In the light of fermentation kinetics, the data from multiple small tests can be sorted and analyzed, and used to guide the design of large-scale fermentation process, thus controlling the production process [13, 14].

The most common application of fermentation kinetics is the study of cell growth using the logistic function. For instance, Lin et al. [10] solved the kinetic parameters and set up a kinetic model for the cell, alcohol and the substrate. Li et al. [8] conducted nonlinear fitting of test data by logistic model, laying the basis for production of jujube wine.

In this test, the potato was fermented with high-activity dry yeast. The logistic function was adopted to describe the growth rate, alcohol yield and total sugar content of the yeast in the fermentation broth. Based on the test data, the author established a fermentation kinetics model, shedding new light on the fermentation of potato liquor.

Materials and methods

Materials and instruments

Raw materials

The raw materials involve potato, corn, sorghum, duck wheat, Angel high-activity yeast, and granulated sugar (premium or first grade). These materials are all provided by the Fermentation Laboratory, Xicang University.

Reagents

The test reagents are 3,5-dinitrosalicylic acid (DNS), standard glucose solution, hydrochloric acid (HCl) solution, iodine-potassium iodide (I-IK) solution, sodium hydroxide (NaOH) solution, phenolphthalein indicators, sucrose solution. All reagents are provided by the Fermentation Laboratory, Xicang University.

Instruments

The test instruments include UV-504 ultraviolet-visible and visible (UV-Vis) spectrometer (Mapada Instruments, China), XMTD-8222 incubator (Jing Hong, China) and ES-44-SM optical microscope (Learning Resources, USA).

Test method

Technological process of potato liquor fermentation test is shown in Fig. 1.

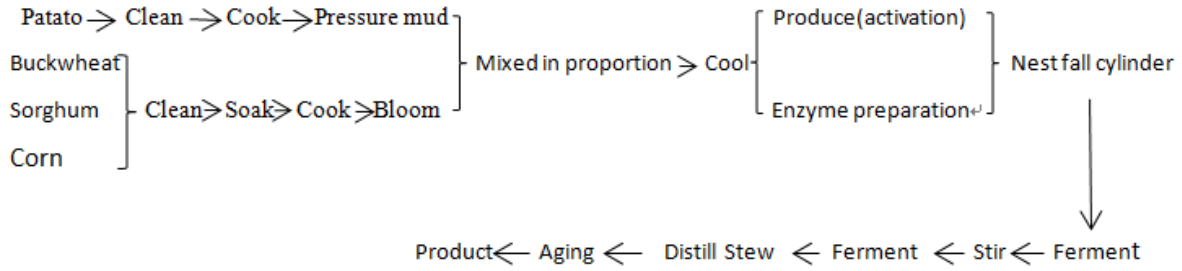

Fig. 1 Potato liquor fermentation test

Key operations are as foloows:

a. Cooking. First, 4.5 kg undamaged potatoes were weighed, cleaned and steamed for 40 min in a pot. The steaming was terminated when the potatoes could be easily pressed down with one hand. The steamed potatoes were taken out of the pot, cooled down to room temperature, and fully peeled and smashed. Next, 1 kg corn, 1 kg sorghum and 1 kg duck wheat were weighed, and cooked in separate pots for 30 min. The cooking was terminated when the skin started to break. The three materials were also taken out of the pots, and cooled down to room temperature. Finally, the four cooled materials were mixed uniformly.

b. Loading into a porcelain jar. The raw materials were mixed uniformly with yeast, and then loaded into a porcelain jar. After that, the surface of the raw material was pressed gently, forming a groove in the middle. A layer of dry yeast was sprinkled on the surface. Next, the jar was covered with a lid, and sealed up with a proper amount of water along the outer edge.

c. Fermentation. The activated yeast was mixed uniformly with the raw materials, and then loaded into a porcelain jar of suitable volume. The jar was relocated into the XMTD-8222 incubator at the temperature of 28-30 ℃. A sample was collected every other day to measure the sugar content, alcohol content, and cell biomass of the fermentation broth.

d. Strain activation. First, 75 g could was weighed and mixed uniformly with 750 g 10% sucrose solution. Under 40 ℃, the mixture was rehydrated for 30 min at 40 ℃, aiming to fully activate the high-activity dry yeast and restore the normal function of most cells.

e. Distillation. First, 500 mL fermentation broth was relocated to a round-bottom distillation flask, and then added with 500 mL distilled water. The flask was heated up with an electric jacket to around 85 ℃. The evaporated alcohol flowed into a conical flask via the condensing reflux tube. The temperature was controlled at 85-90 ℃ throughout the 60 min-long distillation process.

Test methods used are as follows:

1. Yeast cell counting with a hemocytometer

The yeast cells were counted with a hemocytometer. A glass slide with 16 medium chambers (each medium chamber contains 25 small chambers) was selected for the direct count [4, 7, 15]. The fermentation broth was directly dropped on the hemocytometer. Then, the number of yeast cells was observed under the optical microscope. On this basis, the yeast content in the broth was derived.

2. Alcohol content measurement by specific gravity method

According to the *Method for analysis of hygienic standard of distilled wines and mixed wines* (GB5009225-2016), a Chinese national standard for food safety, 500 mL fermentation broth of potato liquor was placed in a beaker, and then distilled in the distillation flask. The fully distilled fermentation liquor was cooled to room temperature, and measured by an alcohol meter. The alcohol content, temperature and volume were all recorded. The conversion formula is as follows:

$$Liquor\ yield = \frac{Alcohol \times Distillation\ volume}{Sample\ quality \times (1 - Moisture\ content\%)} \times 100\%,$$

3. Total sugar measurement by spectrophotometry

According to the *Analytical methods of wine and fruit wine* (GB/T 15038-2006), another Chinese national standard for food safety, 2.0 mL DNS reagent was added respectively in 5 tubes. The tubes were placed in boiling water bath for color development. After two minutes, the tubes were taken out and cooled quickly with tap water. Next, 9.0 mL distilled water was added to each tube, and the mixture was shaken well. After that, the absorbance of the solution was measured at the wavelength of 540 nm using the UV-Vis spectrometer. Meanwhile, the standard glucose solution was replaced with 1.0 mL distilled water, serving as the control group. The control group was subjected to the same color development operations. Finally, the standard curves were drawn with glucose content (mg) as the abscissa and the absorbance as the ordinate.

The fermentation broth was accurately weighed, and poured into a 100 mL conical flask. Then, 100 mL HCl and 15 mL distilled water were added sequentially into the conical flask. Once fully shaken, the conical flask was immediately put in a boiling water bath. After 30 min, 1-2 drops of the solution were taken with a rubber bulb dropper and dropped on a white porcelain plate. The degree of hydrolysis was checked by adding a drop of I-IK solution to the liquid on the plate [1, 2, 11]. If the liquid is not blue, the hydrolysis is complete; otherwise, the flask should be heated in the water bath for more time. After the completion of hydrolysis, the flask was cooled with running water. One drop of phenolphthalein indicator was added to the solution. Then, the solution was neutralized with NaOH solution until it turned red. After that,

the solution was diluted to 100 mL, and filtered with a funnel. 10 mL filtrate was taken, placed in a 100 mL volumetric flask, diluted to 100 mL, and mixed uniformly. The resulting solution was the total sugar hydrolysate diluted 1,000 times, which was adopted for measuring the total sugar content [3, 5, 12, 16, 18].

Fermentation kinetic model

The fermentation kinetics was employed to obtain the physiological features of relevant microorganisms, the growth rate of cells, the optimal conditions for alcohol formation, and the optimal fermentation parameters. These results are critical to the control of fermentation process, design of the fermentation jar and the computer calculation of the fermentation process.

1. Yeast growth model

The yeast growth was described by the logistic model. This model reflects the nonlinear relationship between the cell growth rate and the nutrient content in the fermentation broth. It is very suitable for the study of fermentation kinetics in different batches of materials. The following logistic function was adopted for our test:

$$\frac{dx}{dt} = \frac{\mu_{m1} x}{1 - \frac{x}{x_m}}, \tag{1}$$

where t is time; x – yeast content; μ_{m1} – maximum specific growth rate of yeast; x_m – maximum yeast content. At the beginning of fermentation, $t = 0$ and $x = x_0$. The integral formulas of the above function can be expressed as:

$$\mu_{m1} t = \ln\left(\frac{x_m}{x_0} - 1\right) + \ln\left(\frac{x}{x_m - x}\right) \tag{2}$$

or

$$x(t) = \frac{x_0 \exp(\mu_{m1} t)}{1 - \frac{x_0}{x_m}\left[1 - \exp(\mu_{m1} t)\right]}. \tag{3}$$

2. Alcohol production model

During the fermentation, the amount of alcohol produced depends on the growth rate of the yeast. At the beginning of fermentation, the yeast grows slowly to adapt to the fermentation environment, resulting in a relatively few fermentation product (alcohol). After entering the logarithmic growth phase, the yeast becomes highly metabolic, which gradually pushes up the alcohol production. In the later stage, the yeast growth slows down due to the substrate, and the alcohol production is reduced and stabilized [6, 17]. Obviously, the alcohol production model bears resemblance to the yeast growth model. Hence, our alcohol production model was developed mimicking the yeast growth model. The logistic function for alcohol production model can be expressed as:

$$\frac{dp}{dt} = \frac{u_{m2} p}{1 - \frac{p}{p_m}}, \tag{4}$$

where p is alcohol content; μ_{m2} – maximum production rate of alcohol; p_m – maximum alcohol content. At the beginning of fermentation, $t = 0$ and $p = p_0$. The integral formulas of the above function can be expressed as:

$$u_{m2}t = \ln\left(\frac{p_m}{p_0} - 1\right) + \ln\left(\frac{p}{p_m - p}\right) \tag{5}$$

or

$$p(t) = \frac{p_0 \exp(u_{m2}t)}{1 - \frac{p_0}{p_m}\left[1 - \exp(u_{m2}t)\right]}. \tag{6}$$

3. Substrate consumption model

During fermentation, sugar acts as the carbon source for yeast growth. The reduction of sugar provides the energy needed for the metabolism of the yeast, increases the alcohol content, and produces the sweet substances that improve the taste of potato liquor [9, 19-21]. In our test, the substrate consumption can be expressed as:

$$\frac{ds}{dt} = \frac{1}{y_{x/s}}\left[\frac{d_x}{d_t}\right] - \left[\frac{1}{y_{p/s}}\right]\left[\frac{d_p}{d_t}\right] - k_e x. \tag{7}$$

Substituting (4) into (7), we have:

$$\frac{ds}{dt} = \left(\frac{1}{y_{x/s}} + \frac{a}{y_{p/s}}\right)\frac{d_x}{d_t} - \left(\frac{\beta}{y_{p/s}} + k_e\right)x \tag{8}$$

or

$$\frac{ds}{dt} = -b_1 x - b_2\left[\frac{d_x}{d_t}\right], \tag{9}$$

where s is substrate content; $b_1 = \frac{\beta}{y_{p/s}} + k_e$; $b_2 = \frac{1}{y_{x/s}} + \frac{\alpha}{y_{p/s}}$. At the beginning of fermentation, $t = 0$ and $s = s_0$.

Since $\frac{dx}{dt} = 0$ in the (9) stage, we have: $b_1 = -\frac{\frac{ds}{dt}}{x_m}$.

The following can be obtained through integration:

$$s(t) = s_0 - b_2 A(t) - b_1 B(t). \tag{10}$$

Then, (10) can be rewritten as:

$$A(t) = x(t) - x_0,$$
$$B(t) = \frac{x_m}{\mu_{m1}} \ln\left\{1 - \frac{x_0}{x_m} \times [1 - \exp(\mu_{m1} t)]\right\}. \quad (11)$$

Note: See the Appendix for the meanings of symbols in the formulas.

Test results and analysis

Variation in yeast, alcohol and sugar contents during the fermentation of potato liquor

The test data on fermentation of potato liquor are listed in Table 1. It can be seen that the yeast played an important role in the fermentation test, and the yeast growth exhibited as a typical S-shaped curve with distinct segments. The yeast grew rapidly with short lags. The logarithmic growth phase started on the third day, and the stable phase began on the eleventh day. At the beginning of fermentation, the alcohol production was almost zero, and the sugar content was at the peak. With the growth of the yeast, the alcohol production continued to increase, while the sugar content gradually decreased. According to the growth law of yeast cells, the cells grew rapidly when the substrate was abundant; due to the gradual depletion of substrate, the period between the twelfth day and the fifteenth day saw slow consumption of substrate and relatively small production of alcohol.

Table 1. The parameters in the process of fermentation

Time, d	Number of yeast, $\times 10^7$ cfu/mL	Alcohol yield, %	Sugar content, g/L
0	0.00	0.00	457.11
1	1.25	0.50	451.89
2	2.35	1.30	430.67
3	3.25	2.80	386.53
4	4.00	4.40	318.39
5	4.90	7.20	261.43
6	5.79	10.20	211.86
7	6.30	15.40	165.11
8	6.72	20.40	107.49
9	6.90	24.00	57.92
10	7.08	27.60	25.79
11	7.12	30.10	13.57
12	7.10	30.60	10.96
13	7.11	31.60	5.74
14	7.10	31.50	2.74
15	7.10	31.50	2.23

Kinetics of yeast growth

According to Eq. (2) and Table 1, the slope and intercept of the straight line of $\ln(x/(x_m - x))$ relative to t are respectively b and $\ln(x_m / x_0 - 1)$. Thus, the linear regression equation can be established as:

$y = bx + a$,

where y is $\ln(x/(x_m - x))$; x is t; a is $\ln(x_m / x_0 - 1)$; b is μ_{m1}. Then, the linear regression equation can be obtained as:

$\bar{Y} = 2.0335 + 0.6588x$.

Since $\ln(x_m / x_0 - 1)$ and X_m = 7.12×10^7 cfu/mL, we have X_0 = 0.824×10^7 cfu/mL. Therefore, μ_{m1} = 0.6588 d^{-1} and X_0 = 0.824×10^7 cfu/mL. Substituting these data into Eq. (3), the kinetic model of yeast growth can be derived as:

$$x(t) = \frac{0.824\exp(0.6588t)}{1 - \frac{0.824}{7.12}[1 - \exp(0.6588t)]}.$$

Fig. 2 compares the results simulated by the kinetic model and the measured results. Obviously, the mean relative error between the simulated results and the measured results was 4.3%, indicating that the simulated results agree well with the measured results. As shown in Fig. 2, at the beginning of fermentation, the yeast grew slowly to adapt to the fermentation environment; the yeast growth picked up speed with the elapse of time, and entered logarithmic growth between the third and the ninth day; after the eleventh day, the yeast grew slowly, entering the stable phase.

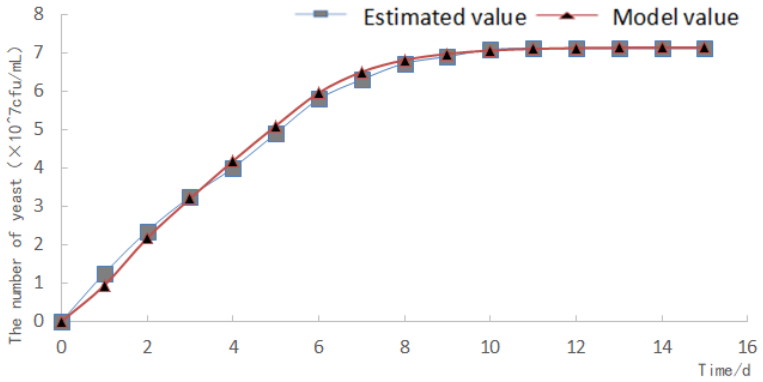

Fig. 2 Comparison between simulated and measured yeast growths

Kinetics of alcohol formation

According to Eq. (5) and Table 1, the slope and intercept of the straight line of $\ln(p/(p_m - p))$ relative to t are respectively μ_{m2} and $\ln(p_m / p_0 - 1)$. Thus, the linear regression equation can be established as:

$y = bx + a$,

where y is $\ln(p/(p_m - p))$; x is t; a is $\ln(p_m / p_0 - 1)$; b is μ_{m2}. Then, the linear regression equation can be obtained as:

$\bar{Y} = 0.6636x + 4.476$.

Since $\ln(p_m / p_0 - 1)$ and P_m = 31.6, we have P_0 = 0.3555. Therefore, μ_{m2} = 0.6636 d^{-1} and P_0 = 0.3555. Substituting P_m = 31.6, μ_{m2} = 0.6636 d^{-1} and P_0 = 0.3555 into Eq. (6), the kinetic model of alcohol formation can be derived as:

$$P(t) = \frac{0.3555\exp(0.6636t)}{1 - \frac{0.3555}{31.6}[1 - \exp(0.6636t)]}.$$

Fig. 3 compares the results simulated by the kinetic model and the measured results. Obviously, the mean relative error between the simulated results and the measured results was 6.01%, indicating that the simulated results are basically consistent with the measured results. As shown in Fig. 3, the alcohol production increased with the fermentation time; the increase rate was maximized from the sixth to the tenth day and ceased to grow after the eleventh day.

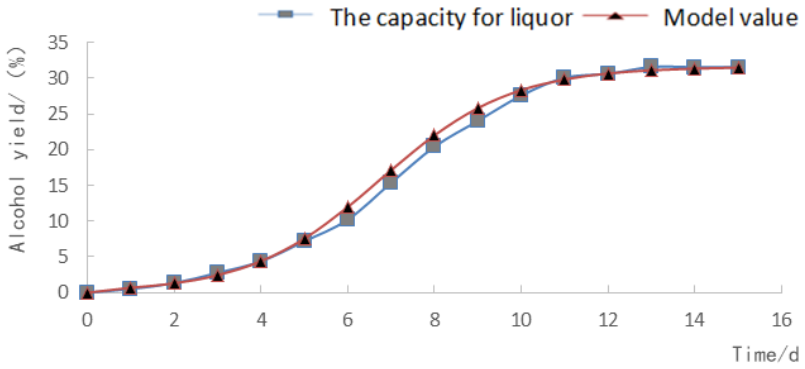

Fig. 3 Comparison between simulated and measured alcohol productions

Kinetics of substrate consumption

According to Eq. (11), the following can be derived from Eq. (10):

$$s(t) = s_0 - b_2(x(t) - x_0) - b_1 \frac{x_m}{\mu_{m1}} \ln\left\{1 - \frac{x_0}{x_m}[1 - \exp(\mu_{m1} t)]\right\}.$$

Based on the kinetic model parameters μ_{m1} = 0.6588 d^{-1} and $x_0 = 0.824\times10^7$ cfu/mL and the test data $x_m = 7.12\times10^7$ cfu/mL and s_0 = 457.11 g/L, a set of equations in two unknowns was established, and the model parameters were obtained as b_1 = 1.93 and b_2 = 17.68. Hence, the kinetic equation for the total sugar consumption can be set up as:

$$s(t) = 457.11 - 17.68(x(t) - 0.824) - 1.93\times\frac{7.12}{0.6588}\ln\left\{1 - \frac{0.824}{7.12}[1 - \exp(0.6588t)]\right\}.$$

Fig. 4 shows the comparison between simulated and measured sugar consumptions. Obviously, the mean relative error between the simulated results and the measured results was 8.22%, indicating that the simulated results are in line the measured results. As shown in Fig. 4, the sugar content changed continuously with the fermentation time; from the fourth day, the sugar was consumed more and more rapidly, providing carbon and energy to yeast growth; after the eleventh day, almost all sugar was consumed, the yeast stopped from growing and the fermentation basically ended.

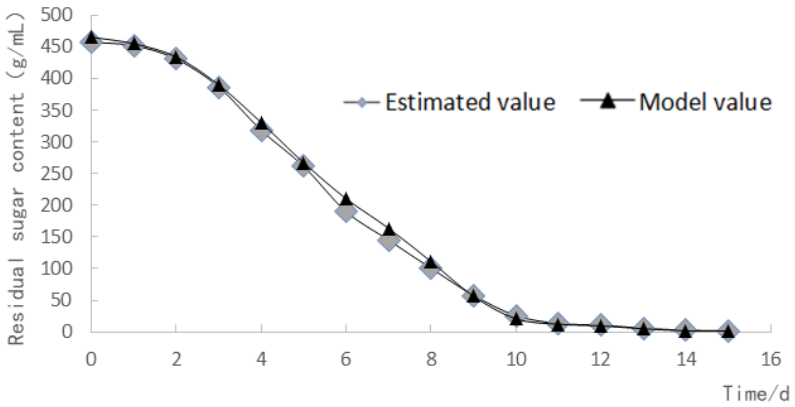

Fig. 4 Comparison between simulated and measured sugar consumptions

Conclusions

Based on logistic function, a series of kinematic models were created for the fermentation process of potato liquor. The results simulated by the models were proved basically in line with the test data, revealing the feasibility of the models.

The test results show that the yeast underwent logarithmic growth from the third to the ninth day. This phase is obviously favorable for the growth in cell content. Starting from the eleventh day, the sugar content almost decreased to zero, and the yeast virtually ceased to replicate. The fermentation kinetics can be applied to the actual production of potato liquor. The kinetic conditions for fast yeast growth and huge alcohol production should be provided to realize process control, optimize production technology and improve production efficiency. The alcohol production can be increased through the control of fermentation process.

The fermentation kinetic analysis provides insights into the physiological features of relevant microorganisms, the growth rate of yeast, the optimal conditions for alcohol formation, and the relationship between related parameters. These results lay a solid basis for the control of fermentation technology.

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Appendix

Formula symbol description:

x_0	represents the initial bacterial concentration, g/L
$x(t)$	represents the concentration of bacteria, g/L
x_m	represents the maximum bacterial concentration, g/L
μ_{m1}	is the maximum specific growth rate, h^{-1}
μ_{m2}	represents the maximum specific rate of production, h^{-1}
p_0	represents the initial alcohol concentration
$p(t)$	represents alcohol concentration
α	represents kinetic model parameters, g/g
β	represents kinetic model parameters, g/(g·h)
s_0	represents the initial substrate concentration, g/L
$s(t)$	is the substrate concentration, g/L
b_1	represents kinetic model parameters, g/(g·h)
b_2	represents kinetic model parameters, g/g
$y_{x/s}$	represents the yield coefficient of bacteria, g/g
$y_{p/s}$	is the product yield coefficient, g/g
k_e	represents the cell maintenance coefficient, h^{-1}
t	denotes fermentation time

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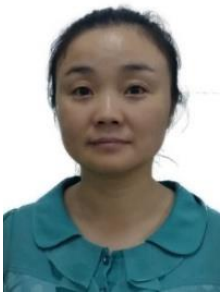

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