0273-2289/96/6003—0229$09.00

Estimation of the Optimal Concentrations of Residual Sugar and Cell Growth Rate for a Fed-Batch Culture of *Saccharomyces cerevisiae*

RONG-QIAO HE,*[,1] CHUAN-YOU LI,[2]
JUNG XU,[2] AND XIU-AN ZHAO[2]

Laboratory of Molecular Neurobiology and Developmental Biology,
[1]Institute of Biophysics, Academia Sinica, Beijing 100101;
[2]Institute of Microbiology, Academia Sinica, Beijing 100080

Received May 27, 1995; Accepted August 9, 1995

ABSTRACT

Estimation of the optimal concentrations of residual sugar in medium for a fed-batch culture of Baker's yeast has been studied and practiced. The concentrations, however, depended on different species and targets of the biomass, which was expected to be made. Kinetic changes of the residual phosphate salt in the medium conformed to a logarithmic process until the fourth hour during an 11-h culture. The parabolic method (*see* ref. *9* later in article) might be qualified to maintain the concentrations of residual sugar around 0.15 g/L. It was demonstrated that cell growth followed a sigmoid process during a fed-batch culture, because the cells consumed the nutrient with two metabolic pathways, one was for cell conversion and another was for non-cell conversion. With the parabolic method, we can estimate kinetics of cell growth and cell growth rate during the culture.

Index Entries: Cell growth; *Saccharomyces cerevisiae;* Baker's yeast; supplementation; cell culture.

Abbreviations: ADY, active dry yeast; GPR, gas production rate; CY, compressed yeast; RQ, respiratory quotient.

*Author to whom all correspondence and reprint requests should be addressed.

Notations: α, cell conversion quotient (liter^2): biomass produced from consumption of 1 g sugar; β, non-cell conversion quotient (liter/g): byproducts produced from consumption of 1 g sugar; μ, specific growth rate (h^{-1}); Ψ, consumption parameter (g^2); x, concentration of residual sugar in medium (g liter^{-1}); V_t, total nutrient consumption rate (v/h); V_i, nutrient consumption rate on reproducible growth (v/h); V_j, nutrient consumption rate by Pasteur effect, Crabtree effect, and production rate of byproducts (v/h); V_c, cell growth rate (g/h); M_c, biomass (g); t, time (h).

INTRODUCTION

Crabtree (*1*) discovered the yeast (*Saccharomyces cerevisiae*) ferments glucose to produce ethanol in media containing sugar of high concentrations, even though ventilation is given. This is called aerobic fermentation or Crabtree effect. It may be due to the catabolite repression, which results from inhibition of the respiratory enzymes by products from sugar. Under this condition, yield of the fermented ethanol increases and reproduction of biomass decreases. Therefore, it is significant to investigate the optimal concentrations of residual sugar in the culture. Recently, Tayeb et al. (*2*) discovered a method for process of alcoholic fermentation of *S. cerevisiae* and demonstrated the optimal concentrations of sugar for fermentation of ethanol. But how to estimate the optimal concentrations of residual sugar for cell growth, not for fermentation of ethanol, is another program concerning both cytology and technology. Previous work (*3*) showed that when concentrations of residual sugar were maintained around 0.000684 g/L, yeast produced little ethanol with the respiratory quotient (RQ) nearly 1.0. With strong ventilation, yeast may not produce ethanol if the residual concentration is less than 0.004 g/L (*3*).

In fact, cell growth is a complicated process, so it is not easy to demonstrate accurately the optimal concentrations of residual sugar. We are obliged to give a range for it. Recently, some methods have been reported that they are mathematical models of fermentation processes which depend mainly on four principles:

1. Ecological and physiological;
2. Ecological and genetic in part;
3. Morphological and organism growth kinetics; and
4. The universally recognized facts of the mutability of organisms (*4*).

The Monod model (*5*) has been widely used in culturing different species (*6*). Mignone and Avignone (*7*), however, described a method of calculating the values of operational variables for a fed-batch culture with a linear gradient feed of nutrients. Xu et al. (*8*) designed a supplementation method with the logarithmic ruler to culture Baker's yeast. He et al. (*9*) has derived a mathematical model for culturing Baker's yeast on the basis

of the instantaneous specific growth rate and sugar conversion rate, as well as on Crabtree and Pasteur effects (*10–14*).

For culture of *S. cerevisiae,* however, qualities of the product, such as GPR and shelf life, are important. To make high quality biomass, we should pay attention to the cell cycle during the culture, for the cycle can be controlled by supplementation (*9*). Cells in G_1 phase are more resistant to drying and aging, features which are particularly important in the process of producing dry yeast.

Like *Amoeba proteus, Tetrahymena macronuclei, Paramecium datum, Paramecium caudatum,* and *Paramecium aurelia* (*15*), the growth of *S. cerevisiae* followed a sigmoidal process during the culture. In other words, changes of DNA, RNA, and proteins of the cells were also observed to follow sigmoid processes (*9,15*). This article is concerned with a model for estimation of cell growth rate and the optimal concentrations of residual sugar, which is needed to culture high quality yield of the biomass.

MATERIALS AND METHODS

Materials

Beet molasses from a local factory contained 54–58% dry materials, 42–48% sugar, and 7–8% ash, after purification with phosphoric acid and lime (*8,16–17*). The strain of Baker's yeast (*S. cerevisiae*), which could be made into ADY, came from The Center of Chinese Biotechnological Corporation. Its gas production rate (GPR) was about 900–1000 mL in compressed yeast (CY). The sulfuric acid and phosphoric acid used were of chemical grade. The other reagents are analytical grade, purchased from local chemical companies and used without further purification.

Medium of Reaction

The scheme of amplification for biomass was according to Xu et al. (*8*). At the initial stage of the culture, a medium of 2400 mL water containing 0.2% sugar and 8.4 g $Na_2HPO_4 \cdot 12H_2O$ and 1.5 g $(NH_4)_2SO_4$ was prepared for inoculation of 1.2% Baker's yeast (in yeast cake; w/v). Beet molasses (580 mL, diluted to contain 20% sugar), with 2.0 g yeast extract and 420 mL water containing 15.8 g $(NH_4)_2SO_4$ and 1.8 g $MgSO_4 \cdot 7H_2O$, were for supplementation by pumps with the parabolic process (*9*). A small quantity of *D*-biotin was added into the molasses before supplementation. Temperature and pH were controlled at 30°C and 4.5, respectively, by running water (18°C) and 10% Na_2CO_3 during the 11 h culture.

Determination of Cells Number

The number of cells was determined by the method of Thomas (*19*). Even though the budded cell was an immature one, an appreciable bud on

the mother cell was identified as a new cell under microscope for determination of the number.

Preparation of ADY and Determination of Shelf Life

The yeast cake was ventilated at 45°C for about 4 h, until the moisture of cells decreased to approximately 9%. The cell mortality was determined by cell staining with 0.2% methylene blue. Shelf life was demonstrated by storing ADY at 43°C for 2 wk. If the residual activity was more than 50% of the original one, it corresponded to a shelf life over 1 yr for the ADY (*8,9,18,20*).

Determination of Dry Weight of Cells

Fifty mL fermentation broth was taken from the fermentor during the culture of desired time intervals and centrifuged (2000*g*, 20 min, 4°C). The precipitated cells were dried in an oven at 105°C for 12 h. After they had been cooled at room temperature in a desiccator, the weights of cells were measured.

Determination of Ethanol

Ethanol in the medium was measured with Shimadzu-7AG Gas Chromatography (Tokyo). Ten mL of the fermentation broth was centrifuged (2000*g*, 4°C, 20 min) and the supernatant was for ethanol measurement on HPGC. Ethanol (0.1%) was used as control. Measurements of GPR (the ability to produce CO_2 from the yeast measured with volume of mL) were according to the Ministry of Light Industry of China, QB596-82, 1982.

Determination of Residual Nitrogen, Residual Phosphate, and Glucose

Determination of the residual nitrogen was carried out on the basis of the method of Micro-Kjeldahl (*21*). Measurement of the residual phosphate was according to Fiske (*22*). A kit of glucose oxidase coupled with peroxidase (GOP-PAP) was purchased from Zhongsheng High-Tech Bioengineering (Beijing) for determination of the residual glucose.

Fermentors and Spectrophotometers

A 6-L fermentor from Pharmacia (Uppsala) was used for experiments in the laboratory. Fermentors with working volume of 36 tons were used to produce Baker's yeast in the breweries. Shimadzu UV-250 and UV-3000 spectrophotometers were used for measurements of absorbance.

RESULTS

Consumption Parameter of Carbon Source

Theoretically, consuming 1 g sugar under an optimal condition, Baker's yeast reproduces 0.567 g of itself with GPR > 800 mL. Hence this cell conversion quotient (α) is 0.567. As we know, the cell conversion quotient is correlated with the concentration of residual sugar in media. That is, each of the metabolic pathways depends upon a certain range of sugar concentration. Besides consumption of sugar for supporting energy and synthesis of the biomolecules, yeast consumes sugar with Pasteur and Crabtree effects (*1–3*), to produce ethanol. For sugar to be consumed by the metabolic pathways, there may be a consumption parameter for each way to elaborate the relationship between the metabolic state and the concentration of sugar in the medium. For this, however, we have obtained an equation by experience and experiments about this relationship:

$$d^2\Psi / d^2x = \alpha \tag{1}$$

and

$$\Psi = \alpha x^2 + \beta x + C \qquad (x \geq 0) \tag{2}$$

where Ψ, α, β and x are, respectively, consumption parameter, cell conversion quotient, non-cell conversion quotient, and concentration of residual sugar, and C is a constant. Cell conversion quotient is defined as yield of the dry yeast reproduced from supplementation of 1 g sugar, and non-cell conversion quotient is byproduct made from supplementation of 1 g sugar. Ψ represents the stability of a metabolic process, which is a function of the concentration of sugar. The higher the consumption parameter increases, the less the metabolic process stability is. Logically, for each nutrient, there should be a consumption parameter to express the relationship between the metabolic state and the concentration of the nutrient. Hence the parameter is related to the optimal concentration of nutrient for the specific metabolic process. For culture of Baker's yeast, the consumption parameter of sugar can be further expressed. As we know, under sufficiently aerobic conditions, the reaction of oxygen with sugar in yeast cells is simplified as follows (*23*):

$$C_6H_{12}O_6\,(\text{glucose}) + 6O_2 \rightleftharpoons 6CO_2 + 6H_2O \tag{3}$$

As stated, if 1.0 g sugar is supplemented to the medium and 0.567 g sugar converted to biomass, we calculate the converted sugar on the left side of Eq. (3), with CO_2 and H_2O at gaseous state, since the sugar will eventually be converted into CO_2 and H_2O. Consequently, 0.0189 mol H_2O and CO_2 would be produced respectively ($6 \times 0.567/180^* = \text{g/g/mol} = \text{mol}$), where * = molecular mass of glucose. The volume of the gases (CO_2 and H_2O) per mol should be 22.4 at 298 T and 1 atm. Therefore, $\alpha = 0.717$ L^2 [$(2 \times 0.0189 \times 22.4)^2 = (\text{mol} \times \text{L/mol})^2 = L^2$].

On the other hand, yeast ferments glucose to produce ethanol, on the basis of Crabtree effect. The reaction equation is expressed as follows:

$$\text{glucose} \rightleftharpoons 2C_2H_5OH(\text{ethanol}) + 2CO_2 \quad (4)$$

Because the optimal cell conversion quotient for production of Baker's yeast is 0.567, non-cell conversion quotient should be 0.433 g, this is to say 0.00481 mol ethanol and carbon dioxide calculated by Eq. (4) were produced respectively. $0.00481 \times 22.4 \times 2 = 0.215$ L (mol × L/mol = L). This is produced from consumption of 1.0 g of the carbon source. Hence, $\beta = 0.215 \text{ L} \times 1.0 \text{ g} = 0.215$ (L/g). Here we suppose β to be negative.

$$\Psi_{\text{yeast}} = 0.717x^2 - 0.215x + C \qquad (x \geq 0) \quad (5)$$

On the basis of Eq. (5), the dimension of Ψ_{yeast} should be g^2. Ψ_{yeast} always increases while the residual sugar is more or less than the optimal concentration. In fact, x should not be negative, because x represents the concentration of residual sugar.

The Optimal Concentrations of Residual Sugar

The optimal concentrations of sugar should be different, depending on different species and targets of the final products. For example, if we had wanted to produce ethanol, we would have supplemented more sugar to maintain the concentrations higher than those for production of biomass. On the other hand, when we planned to produce the biomass, we could calculate the proper concentration from Eq. (5).

When

$$d\Psi_{\text{yeast}} / dt = 0 \quad (6)$$

the optimal concentration of residual sugar can be determined. Relationship between the optimal concentration of residual sugar and cell conversion quotient is shown in Fig. 1. On the basis of Eq. (6), the optimal concentration of sugar should be 0.00114 g/L if we assume to obtain 0.001 g sugar used for non-cell conversion. This concentration is similar to that (0.00117 g/L) reported by Gorts with experiments in 1967 (*3,24*). Theoretically, if cell conversion quotient is 1.0, the concentration of residual sugar would be zero. In contrast, if the quotient is zero, the concentration would be 100%. In fact, the yeast does not grow at either the concentration 0 or 100%.

For the other production targets, it might be 4.46 g/L, if we attempt to keep cell conversion quotient at 0.2. But for production of Baker's yeast, we have to pay attention to the qualities of the biomass, such as GPR, shelf life, and yield. If the concentration of residual sugar is too low, GPR and yield of biomass will be low (*8–9*).

Nutrient Consumption Rate and Cell Growth Rate

As we know, the best cell conversion quotient for Baker's yeast is 0.567 (*8–14*), and the optimal concentration of residual sugar should be

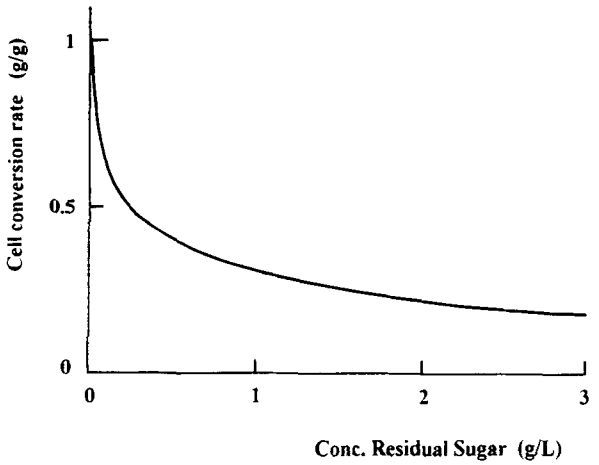

Fig. 1. Relationship between concentrations of residual sugar and cell conversion rate in consumption of 1 g sugar. All figures were calculated on the basis of Eq. (6), under a standard condition (1 atm and 298 T).

kept at 0.15 g/L in the medium. Concentrations of residual sugar which were more or less than 0.15 g/L would affect the quality and yield of the yeast, on the basis of Eq. (6). Hence, the method to control the supplementation rate of sugar to the growing cells becomes imperative. Before describing the method, let us consider the metabolic processes of nutrients and the kinetics of cell growth. According to our previous work (*8*), consumption of nutrients by cells underwent two metabolic pathways: one was for the formation of biomass and the nutrients existing in cells; another was not, and the substances were discharged out of the cells. We could determine the total nutrient consumption rate which included nutrient consumption rate on cell growth and consumption rate on non-cell conversion. The nutrient consumption rate on non-cell conversion was caused by Pasteur and Crabtree effects and production of byproducts. Hence nutrient consumption rate on cell growth may be expressed as follows:

$$V_i = V_t - V_j = n\,(\mathrm{A}\,e^{\mu t + T} - \mathrm{B}e^{\beta t + U} + \mathrm{C}) \tag{7}$$

where T and U represent corrections and A, B, and C are constants. Determination of A, B, T, U, and C is according to He et al. (*9*). The dimensions of V_i, V_t and V_j are vol per h. This volume is that of molasses containing 20% sugar. This definition of dimension is convenient for use when culturing and producing Baker's yeast and n (g) is mass of the inoculated cells, which is calculated into active dry yeast. Now that V_i represents the sugar consumption rate on cell growth, cell growth rate during a fed-batch culture can be expressed as follows:

$$V_c = kV_i \tag{8}$$

where k is a constant for conversion of the volume of molasses into mass (g). On the other hand, when using the volume of cells to show the growth

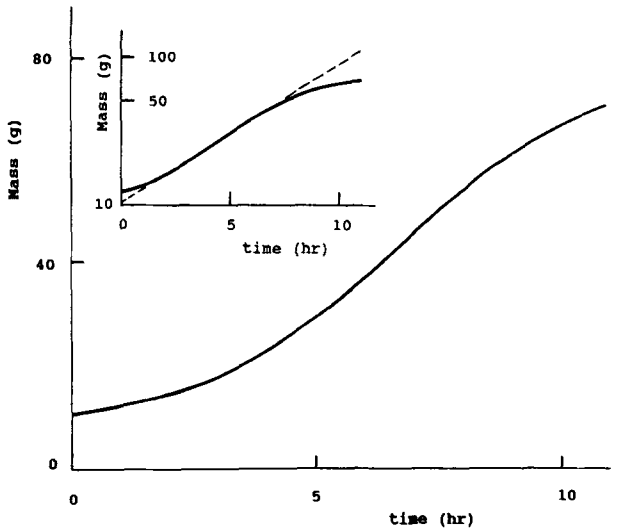

Fig. 2. Kinetics of cell growth in a fed-batch culture. The curve, which represents kinetics of cell growth, is obtained by integration of Eq. 7, similar to that during a fed-batch culture experimentally (*8*). The inset is the same data shown in the semilogarithmic plot.

process, we can calculate the volume of molasses for cells with a constant of 20%, then multiply 1.2 mL/g, because one gram dry yeast occupies approx 1.2 mL vol.

Kinetics of Cell Growth

Kinetics of cell growth can be elaborated by integrating Eq. 7 (Fig. 2). Meanwhile, it can be also determined by measurements of the biomasses at the desired time intervals during the culture. Equation (9) describes changes of biomass during an 11 h fermentation as follows:

$$M_c = \int V_{cdt} = k_n \int (Ae^{\mu t} - Be^{\beta t} - Ct - D)d_t \quad (9)$$

where A, B, C and D are constants (*9*). Equation 9 represents a sigmoid curve. Cell growth has an initial lag phase of about 2 h and an exponential reproduction (3–8 h); a stationary growth followed. This theoretical model corresponds to the experimental process of cell growth observed during the culture of Baker's yeast. The lag time is due to the inoculated cells which are unbudded (in G1 phase). They need time to synthesize DNA, RNA and proteins in preparation for mitotic divisions. We did not observe that the cells were budding during the initial period of 2 h. Then the cells reproduced themselves in an exponential process between 3–8 h. Three generations of the cells might be reproduced during the whole culture. Similar to the kinetics of growth process, V_j follows an exponential process simultaneously (Fig. 3). The V_j is much smaller than V_t during the initial period of the culture, but it becomes larger rapidly with the passage of time. $V_t - V_j$ results in a parabolic process of nutrient con-

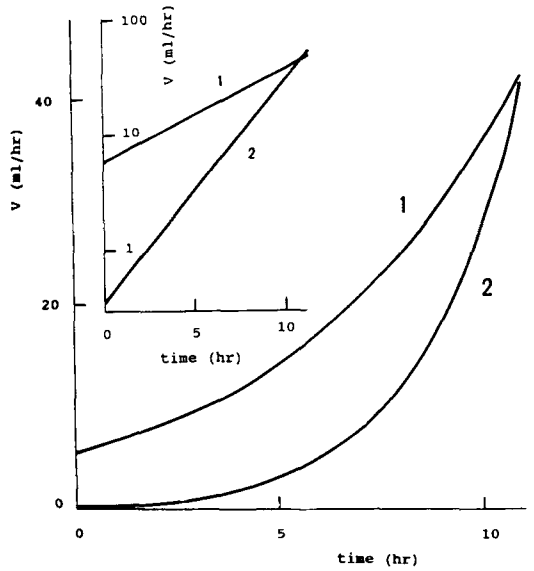

Fig. 3. Changes in supplementation rate and the byproducts production rate. The theoretical supplmentation rate is based on that cell growth follows an exponential process. On the basis of the average specific growth rate in a 10 h culture (*8–14*), the total sugar consumption rate conforms to an exponential process ($V_i = 5.77e^{0.18t}$; curve 1) and the byproduct production rate also follows the same style process ($V_j = 0.36e^{0.433t}$; curve 2). The insets are the same data shown in the semilogarithmic plot.

sumption rate on cell growth (*9*). This parabolic method has been used for culture of Baker's yeast. In fact, it is qualified for culturing yeast.

Cell Growth and Byproducts

During the fed-batch culture, yields of both cells and byproducts increase simultaneously. Theoretically, Baker's yeast should grow following an exponential process with an average specific growth rate of 0.18 (Fig. 3). However, parts of the nutrient have to be used for non-cell conversion. Kinetics of production of byproducts also follow an exponential process (Fig. 4), this yield is very small during the initial period of the fed-batch culture but increases rapidly after 8 h. The process of cell growth undergoes a sigmoid curve, although both kinetics of total consumption of sugar and that of consumption on non-cell conversion conform to exponential processes.

Nutrient Supplementation Rate Derived by Cell Growth Rate

Cell mass per 100 mL fermentation broth was determined at time intervals during the culture (*9*). Equation 9 could be used to simulate kinetics of cell growth and the coefficients of A, B, C, and D can also be

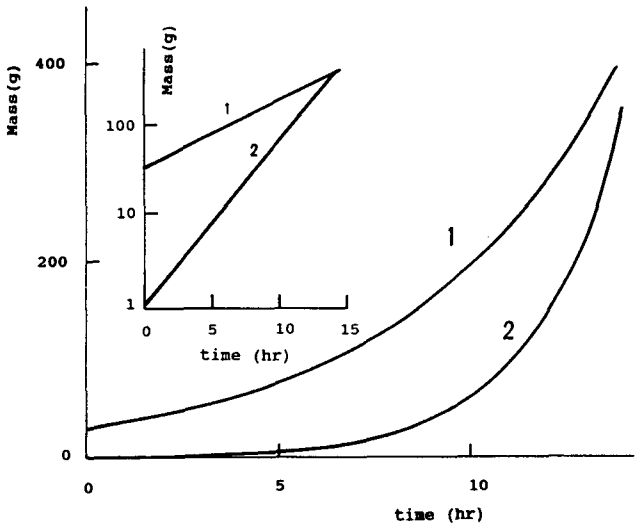

Fig. 4. Changes in consumption mass and byproducts mass. The sugar consumption in mass follows an exponential process (Ms = $32.06e^{0.18t}$) during the culture (curve 1). Changes of the byproducts in mass also conform to an exponential one (Mb = $0.825e^{0.433t}$; curve 2). Changes of the nutrient consumption on cell growth in mass is the difference between Ms – Mb.

determined by experiments and experience. After determination of the expression simulated to the process of cell growth, we derived the equation and get a parabolic expression, which could be used for the nutrient supplementation. Consequently, the parabolic expression could also be used to express the cell growth rate.

$$dM_c / d_t = V_c = kV_i \quad (10)$$

It is convenient to determine cell growth rate and nutrient consumption rate on cell growth with this derivative equation, although some coefficients of the equation should be corrected to correspond to different species under different conditions. Then this supplementation equation may be used to culture the species efficiently.

Changes in Residual Sugar, Nitrogen, and Phosphate

Quality and yield of yeast are dependent upon concentrations of residual sugar in media (Table 1). If the concentrations are lower than 0.01 g/L during the whole culture, GPR of the yeast will not be qualified for bread production. If they are higher than 5 g/L, much more sugar will be fermented to produce ethanol, which would not only waste the carbon source, but also degrade the shelf life of the biomass. It is important to maintain the optimal concentrations of residual sugar in media during the culture. The parabolic supplementation has been used for this purpose and it can control the concentrations of ethanol fermented around 0.1% during the fermentation (Fig. 5).

Table 1
Yields of Ethanol and Biomass
Correlated to Concentrations of the Residual Sugar

Sugar concentration	0.0001	0.0010	0.0100	0.1000	1.0000	(%)[a]
Ethanol yield	0.0000	0.0005	0.0024	0.0460	0.6400	(%)[b]
Biomass yield	2.8	3.2	4.2	5.2	5.3	(times)[c]
GPR	<500	<500	>600	>800	>900	(mL)[d]

These are averages from 12 experiments.

[a]Concentrations of residual sugar were measured with the method of GOD-POD, and the kit of the enzyme reagents was from Beijing Zhongsheng High-Tech Bioengineering Company.

[b]Concentrations of ethanol in the medium were determined by high pressure gas chromatography with GC-7AG at 25°C. It must be noticed that this yield was just ethanol concentration in media, because parts of the alcohol were released into the air by ventilation.

[c]The strain of ADY was purchased from The Center of Chinese Biotechnological Corporation. The yeast (1% of the medium; g/100 mL) was inoculated into the fermenter (6 L, LKB) at 30°C and pH 4.5. The initial medium contained 0.5% $(NH_4)_2SO_4$, 0.16%, $Na_2HPO_4 \cdot 12H_2O$, 0.01% $MgSO_4 \cdot 7H_2O$, and the other nutrients. The parabolic method (*8*) was used for supplementatioan of glucose in an 11 h culture. The final fermentation broth was centrifuged (2000*g*, 25°C, 20 min) and the yeast cake was weighed (g) for determination of the yield of biomasses.

[d]GPR was measured with the method of Xu et al. (*14*).

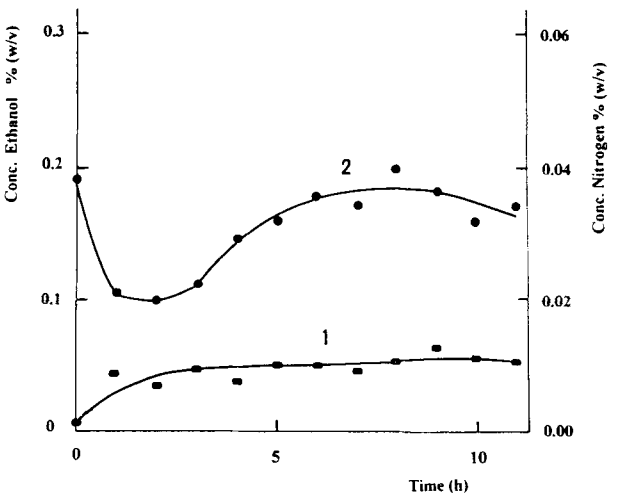

Fig. 5. Concentrations of residual nitrogen and production of ethanol in medium. The supplementation follows Eq. (7). A fermentor of 6 L was employed. The supplementation nutrients were divided in two, the first part containing $(NH_4)_2SO_4$ and $MgSO_4$ and the other containing 20% glucose with biotin and *meso*-inositol. Both of them were supplemented into the fermentor. $Na_2HPO_4 \cdot 12H_2O$ was dissolved in the medium before inoculation of the yeast. Ten mL of fermentation broth was taken from the fermentor and centrifuged (2000*g*, 4°C, 20 min) at desired time intervals for measurements of ethanol (curve 1) and nitrogen (curve 2).

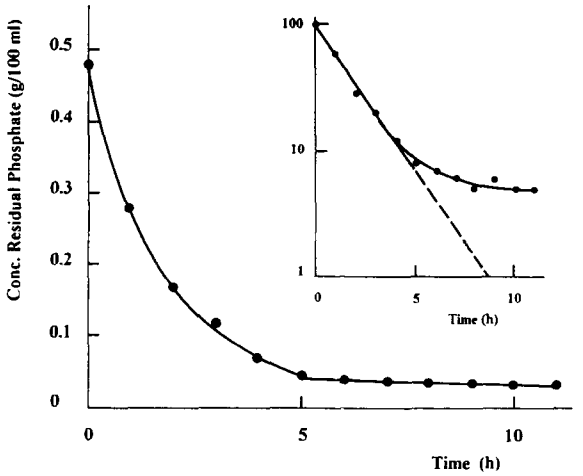

Fig. 6. Kinetics in concentration of residual phosphate in medium during the culture. Conditions were as for Fig. 5, except for measurements of residual phosphate in medium at desired time intervals. The inset is the same data in the semilogarithmic plot.

Changes in residual phosphate in the medium conform to a logarithmic process until the fourth hour, because it was added and dissolved in the medium before the yeast cells were inoculated (Fig. 6). Here we observe that changes in concentrations of the residual phosphate did not follow a complete logarithmic process during the whole culture. The reasons for this may be that 1) the amount of the inoculated yeast is large (1–1.2% in yeast cake; w/v) and the cells could not keep the reproduction of themselves in logarithmic process during the whole culture; 2) some cells did not join mitoses division after the first budding; and 3) some substances produced inhibited the cell growth (7). This suggests that the supplementation of nutrient should not conform to a logarithmic process, but to a parabolic one. As we know, phosphate is one of the sources used for synthesis of DNA and RNA, and many proteins need it to carry out their functions. It appears that if the concentration of residual sugar was maintained around 0.15 g/L, and the other conditions, such as strain of yeast, sterilization, ventilation, molasses, and so on, were also used optimally, the Baker's yeast produced would be qualified.

Features of the Products

Determination of concentrations of residual sugar with the method of GOD-POD showed that they were around 0.15 g/L under the parabolic supplementation until the ninth hour of the culture (Table 2). With this supplementation, GPR and shelf life of Baker's yeast were qualified with a low yield of ethanol to save a certain amount of carbon source.

For preparation of ADY, the yeast cake was placed at 43–45°C for dehydration of cellular moisture. Through thermal dehydration, the moisture decreased from 70% to 9%, with a cell mortality lower than 5%.

Table 2
Specifications of ADY Made by Control of the Concentration of Residual Sugar Around 0.15 g/L in an 11 h Fermentation*

Mortality after dehydration[a]	3–5%
Ethanol concentration during fermentation[b]	0.01–0.3%
GPR of product[c]	950–1000 mL
Shelf life[d]	1–2 yr
Budded cells of product	3–5%

*These are averages from 18 experiments.
[a]The compressed yeast (CY) was placed at 45°C with ventilation, for 4 h. The moistures of cells decreased from 70% to 8%.
[b]The fermentation broth (10 mL) was centrifuged (2000*g*, 4°C, 20 min) and the supernatant was for measurement of ethanol with high pressure gas chromatography.
[c,d]It is according to Xu et al. (*14*).

DISCUSSION

Concentrations of residual sugar in media are not only correlated to characteristics of cell growth, but also to features of cells. One reason for this may be that the metabolic processes depend on concentrations of sugar. In some sense, different sugar concentrations make different targets of the product. For example, Tayeb et al. (2) designed a method for fermentation of sugar to produce ethanol with *S. cerevisiae*. The concentration of sugar they used in the medium was 130 g/L at the beginning of culture. Under this condition, the cells were engaged in fermentation of ethanol. However, when concentrations of sugar were more than 200 g/L, fermentation of ethanol was repressed. In contrast, when the concentrations were less than 1 g/L, repression of ethanol production could be observed under ventilation. We have designed a method for production of the yeast cells, not for fermentation of ethanol. It controlled concentrations of sugar around 0.15 g/L to repress the fermentation. Similar to fermentation of ethanol, when concentrations of sugar were more than 5 g/L, more carbon source was used for the fermentation; when they were less than 0.01 g/L, the nutrient was deficient for Baker's yeast growth during the reproduction. In general, any nutrient, though required by cells, has an optimal range of quantity for a special metabolic process. If the nutrient is either excessive or deficient in the medium, the metabolism concerned will be disturbed (*25*). Though the optimal concentrations depend on different targets to produce, they are dependent upon the conditions which are used in the culture, such as strain of yeast, ventilation, culture time, kinds of carbon sources, and the amount of cells inoculated.

Since the concentrations of sugar are correlated to quality and yield of the biomass, determination and control of the concentration become imperative. On this basis, we introduce the consumption parameter (Ψ_{yeast})

for determination of the optimal concentrations of residual sugar in medium for the culture of Baker's yeast. According to Eq. (5), the consumption parameter should be a parabolic function. Conveniently, estimating the optimal concentrations of residual sugar determines the minimum of the function (when $d\Psi_{yeast}/dx = 0$, there is a minimum). This is to say, when any concentration of residual sugar makes the minimum value of the consumption parameter, the concentration is thus optimal to culture biomass. The significance of the consumption parameter may be: to elaborate a relationship between consumption of nutrient and cell growth; to expound the equilibrium state of the metabolism kept by a proper concentration of the nutrient; and to be used as one method for determination of the optimal concentration of a nutrient.

We have supposed the optimal concentration of residual sugar to be 0.15 g/L, but it is impossible to keep it constant during the culture (0–9 h). This involves the method of nutrient supplementation. Baker's yeast has Pasteur and Crabtree effects, of which the former is related to ventilation and the latter to concentration of sugar. Ventilation is used throughout the culture, so we are concerned about concentrations of sugar. As we know, when the concentrations are lower than the optimal range (*1–2, 8–9,14*), GPR and yield of cells will be lower. When the concentrations are higher, yield and quality of cells produced will become worse for use as commercial product. Meanwhile, increasing the fermentation rate wastes a large amount of sugar to produce ethanol (*26–27*). The proper nutrient supplementation is what the growing cells just require. This is to say, the supplementation should be qualified to produce the biomass with GPR ≥ 800 mL, yield of ethanol during the culture $\leq 0.2\%$, shelf life > 1 yr, cell conversion quotient ≥ 0.5, and yield of biomass ≥ 5 times to that of the inoculated cells. Practically, it is too difficult to control the supplementation rate to feed yeast in such an optimal biochemical and technological condition. To simulate the condition by our experiments and experience, however, the parabolic supplementation should be recommended (*9*).

It is known that an equation for cell reproduction follows an exponential phase expressed by $\lg X = \lg Xo + n \lg 2$ (X represents the number of the cells at any time, Xo represents the initial number of cells and n is generations). This is an ideal equation for expression of cell growth, but it should not be easily used for the culture of any species, since cell reproduction has lag, exponential, stationary, and decline phases (*28*). In fact, it is impossible for cells to divide at the same time, with a synchronous rate of 100% (*29*). There is evidence that kinetics of cell growth follows a sigmoid process (*15*). Moreover, growth of Baker's yeast, as we have seen, follows a sigmoid process. The process is demonstrated by either experiments or calculation. That kinetics of cell growth can be determined by measurements of the biomasses in time intervals during a culture. We could stimulate the kinetic process with Eq. (9). The consumption rate on cell growth and cell growth rate are conveniently derived by the simulated

equation. Although changes in biomass involve some dead cells, they are as low as 0.1% of the viable cells during an 18 h culture (*30*).

Nevertheless, in fact, we should not keep concentrations of residual sugar around 0.15 g/L throughout the culture, because we must control the cell cycle by decreasing the concentration less than 0.15 g/L, to repress the cell division. At the last h of the culture, the concentration of residual sugar should be maintained at less than 0.001/L to make the cells stay in G1 phase (*9*). Otherwise, a large part of cells produced may become budded ones. When making ADY, the budded cells were dead during the cellular dehydration at 45°C for 4 h. Cells that stayed in G1 phase could survive dehydration. During this dehydration, the moisture of cells decreased markedly, followed by changes in metabolic processes of carbohydrate, proteins, ions, and RNA.

ACKNOWLEDGMENTS

This article is dedicated to Prof. Hao Xu and Prof. Hui-Xiu Jiang. We thank Dr Ming-Dong Yang, Ms Yue-Xian Kang, Mr Yong-Hai Ren, and Ms Cong Xiao for their help of this work.

REFERENCES

1. Crabtree, H. G. (1929), *Biochem. J.* **23,** 536–545.
2. Tayeb, A. M., Ashour, I. A., and Mostafa, N. A. (1991), *Energy Convers. Mgmt.* **32,** 491–497.
3. Chen, S. Y. and Xiao, X. P. (1990), in *Biochemistry of the Yeast,* 1st Ed., Du Xiu-Ming and Shi Hong-Ying, eds., Academic Press of Shan Dong, Ji-Nan City, pp. 155–178.
4. Fredrickson, A. G., Megee, R. D., and Tsuchiya, H. M. (1970), in *Advances in Applied Microbiology,* vol. 13, Perlman, D., ed., Academic, New York, pp. 419–464.
5. Monod, J. (1949), *Ann. Rev. Microbiol.* **3,** 371–1949.
6. Fang, B. S. and Lin, J. Q. (1992), *Chin. J. Biotechnol.* **8,** 283–287.
7. Mignone, C. F. and Rossa, A. C. (1993), *Proc. Biochem.* **28,** 405–410.
8. Xu, H., Kang, Y. X., Jiang, H. X., Chen, X. D., Wang, L., and Zhou, J. (1989), *Sci. Sin.* **32,** 822–829.
9. He Rong-Qiao, Xu Jung, Li Chuan-You and Zhao Xiu-An (1993), *Appl. Biochem. Biotech.* **41,** 145–155.
10. Oura, E., Suomalanen, H., and Viskari, R. B. (1982), *Fermented Food,* vol. 7, Rose, A. H., ed., Academic, London, pp. 87–146.
11. Reed, G. and Peppler, H. J. (1973), *Yeast Technology,* AVI, Westport, CT, pp. 103–164.
12. Reed, G. (1982), in *Prescott and Dunn's Industrial Microbiology,* AVI, Westport, CT, pp. 593–634.
13. Reiff, F. (1962), *Die Hefen Bans II. Technologie Der Hefen.* Verlag Hans Carl, Nurnberg, Backhe-fefabrikation, pp. 501–610.
14. White, J. (1954), in *Yeast Technology,* Chapman and Hall, 1st ed., Jarrold, London, pp. 13–27.
15. Mazia, D. (1961), in *The Cell Biochemistry, Physiology, Morphology,* Brachet, J. and Mirsky, A. E., eds., Academic, New York, pp. 98–111.

16. Park, S. C. and Baratti, J. (1991), *Appl. Microbiol. Biotechnol.* **35,** 283–291.
17. He, R. Q., Zhang, G. J., and Yao, J. L. (1995), *Biochem. Mol. Biol. Int.* **36,** 1–12.
18. Xu, H. and Kang, Y. X. (1985), *Microbiology Acta Sinica* **12,** 187–190.
19. World Health Organization (WHO) (1980), in *Manual of Basic Techniques for a Health Laboratory,* Geneva, pp. 360–366.
20. Felsher, A. R., Koch, R. B., and Lorsen, R. A. (1955), *Cereal Chem.* **32,** 117–124.
21. Ballentine, R. (1957), in *Methods in Enzymology,* vol. 3, Colowick, S. P. and Kaplan, N. O., eds., Academic, New York, pp. 984.
22. Fiske, C. H. and Subbarow, Y. P. (1925), *J. Biol. Chem.* **66,** 375–381.
23. Wang, D. I. C., Cooney, C. L., Demain, A. L., Dunnill, P., Humphrey, A. E., and Lilly, M. D. (1979), in *Fermentation and Enzyme Technology,* John Wiley, New York, pp. 57–97.
24. Suomalainen, H. and Oura, E. (1971), in *The Yeast,* vol. II, Ross, A. H. and Harrison, J. S., eds., Academic, London, 271–309.
25. He Rong-Qiao (1995), *Exploration of Nature* **14,** 40–46.
26. Alberta, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J. D. (1983), in *Molecular Biology of the Cell,* Garland, New York, pp. 611–672.
27. Ambrose, J. J. and Easty, D. M. (1971), in *Cell Biology,* Thomas Nelson, London, pp. 289–290.
28. Edwards, C. (1981), The microbial cell cycle. *Lecture in Microbiology at the University of Liverpool,* Hong Kong, 1st ed., pp. 4–10.
29. Nord, F. F. and Weiss, S. (1958), in *The Chemistry and Biology of Yeast,* Cool, A. H., ed., Academic, New York, pp. 323–362.
30. Morris, E. O. (1958), in *The Chemistry and Biology of Yeast,* Cook, A. H., ed., Academic, New York, pp. 251–317.