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The Principle of Parabolic Feed for a Fed-Batch Culture of Baker's Yeast

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ABSTRACT

A parabolic nutrient supplementation has been derived by the specific growth rate, sugar conversion rate, and Pasteur effect, followed by high qualitative and quantitative biomass production with the lowest sugar consumption. The method produced about 95% cells in the G1 phase that were more resistant to drying and aging. These features are particularly important in the process of making dry yeast. It appears that the parabolic feed method may be used to species culture that show the Pasteur effect or produce byproducts from sugar. This may be because the supplementation is in conformity with reproducible kinetic growth during the fed-batch culture.

Index Entries: Baker's yeast; fed-batch culture; specific growth rate; sugar conversion rate.

INTRODUCTION

Making large quantities and high qualities of yeast (*Saccharomyces cerevisiae*) is an important goal of both cytology and technology. The ADY (active dry yeast)-making process is the only example of microbial technology in which thousands of tons of dehydrated active biomass are produced. Reed (*1*) called it a technical triumph. The kinetic consumption of carbon, nitrogen, and other nutrients by cells is one important criterion for nutrient feed in yeast culture. Hayduck and Sak derived a fed-batch

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process to improve the quality and yield of yeast as compared to the Vienna process (*2–6*). The Komax process uses ethanol concentration to control the nutrient supplementation in the waste gas released from medium (*7*). A logarithmic feed for a fed-batch culture of Baker's yeast reported by Xu et al. (*8*) was derived with a slide rule to simulate changes of the instantaneous specific growth rate and corrected by multiple experiments, demonstrating that the sugar conversion rate, shelf life (*8–10*), GPR (gas production rate), and biomass yield were satisfactory. The cell number was fundamental for deriving a method of supplementation, however, it might not be a unique kinetic parameter, since cells in different cycle periods carry out different metabolic processes (*11*). If there is unequal division in *Saccharomyces cerevisiae*, it suggests that the growth, not the event of the DNA division cycle, would limit the rate of cellular proliferation and that the attainment of a critical cell size is a prerequisite for the "start" event in the DNA-division cycle (*12–13*). On the basis of this, it was difficult to derive a nutrient supplementation rate that only conformed to the changes of cell number, since the young cell and the adult cell are not distinguishable under the microscope. Subsequently, Pasteur and Crabtree effects should be considered in deriving the nutrient supplementation model. Maitra and Lobo (*14–15*) reported that sugar increased the specific activities of some glycolytic enzymes, such as D-glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase, and triose-P isomerase, which might increase the metabolism of sugar and cell growth under appropriate conditions. This paper is concerned with the equations for supplementation of carbon, nitrogen, and other nutrients derived from specific growth rate, sugar conversion rate, and the Pasteur effect of yeast in a fed-batch culture. These parameters lead to high quality and quantity of the produced biomass.

MATERIALS AND METHODS

Diphenylamine and orcinol were from Fluka (Buchs, Switzerland) and biotine from Sigma (St. Louis, MO). Beet molasses from a local sugar factory contained 54–58% dry materials, 42–48% sucrose, and 7–8% ash content after purification with phosphoric acid and lime (*8*). The strain of Baker's yeast (*Saccharomyces cerevisiae*) that could be made into ADY came from a Chinese local food factory, whose GPR was about 900–1000 mL in compressed yeast (CY). Sulfuric acid and phosphoric acid were chemical grade. All remaining analytical reagents used to determine proteins, DNA, and RNA were made by the Beijing chemical companies and used without further purification.

The amplification scheme for the biomass was from Xu et al. (*8*). The number of cells was determined by the method of Thomas (*16*) and the immature cells were identified as an appreciable bud on the mother cell under the microscope. Shelf life was determined by storing ADY in a

43°C oven for 2 wk with residual activity >50%. This corresponds to a shelf life over 1 yr for the ADY (*8–9*).

Dry Weight of Cells

Fifty mL fermentation broth from the culture was centrifuged (5000 rmp, 20 min, 4°C) at desired time intervals. The precipitated cells were dried in an oven (12 h, 105°C), and weighed after the temperature decreased to 25°C in a desiccator.

Determination of DNA

Five mL of 0.25*N* $HClO_4$ was added to the centrifuged cells, stirred (10 min, 0°C), and the supernatant was discarded after centrifugation. Four mL of 0.25*N* $HClO_4$ were added to isolate DNA (15 min, 70°C). The product was separated by centrifugation. The next supernatant was used for determination of DNA (*17–18*) after further addition of 3-mL aliquots of 0.25*N* $HClO_4$.

Determination of RNA

Two solutions were prepared as follows:

1. 20 mM EDTA-Na_2, 0.1*M* NaAc, and 1.0% SDS
2. Saturated phenol with water.

One g compressed yeast was mixed with triple 1 and double 2 (mL), stirred (2 h, 45°C) before centrifugation. To this was added 50 mL 95% ethanol precipitated RNA in the supernatant centrifuged at 10000 rpm (20 min, 0°C). The isolated RNA was determined (*19–21*).

The cellular nitrogen and the residual in media (*22–23*) as well as the residual phosphate (*24–25*) were determined at desired time intervals. At the same time, ethanol concentration in the media was measured by Shimadzu-7AG Gas Chromatography. Determination of trehalose was according to Trevelyan (*26*) and GPR measurement was referred to The Ministry of Light Industry of P.R. China, QB596-82, 1982. The produced Baker's yeast was used in local breweries of China. A 6-L fermenter from Pharmacia (Piscataway, NJ) was used in the laboratory. 36-ton fermenters were used in the brewery. Shimadzu UV-250 and UV-3000 spectrophotometers were used for the measurement of absorbence.

RESULTS

Logarithmic Feed

The budding rate of the commercial yeast must be less than 5%; in another words, the yeast should be in the G1 phase, for high viability and long storage. Meanwhile, cells inoculated to the final amplification should

be also in the G1 phase, to protect from random budding of cells. This is controlled by the supplementation rate during the last amplification. The cells were bred at a synchronous ratio (around 62%) in their first division, resulting in young, adult, and aging cells surviving together. To study their reproducible growth kinetic, the instantaneous specific growth rate has been introduced.

$$\mu = (1 / N\,(dN / dt) \tag{1}$$

where μ is the instantaneous specific growth rate, N is the number of cells, and t represents time. It has been demonstrated that the sugar conversion rate (α) is theoretically produced from 1 g sugar made out of 0.567 g biomass (*1–2,4–5,8,27*).

$$\alpha = (M_c) / (V_t) \tag{2}$$

where M_c represents the cell growth rate (g/h) and V_t represents consumed sugar rate (g or mL/h). However, M_c represents the calculated number of cells:

$$\alpha V_t = r N \tag{3}$$

where r is a constant. If α and μ are considered as constants and A instead of ($\mu\, r\, N_o/\alpha$), V_t could be written as:

$$V_t = A\, e^{\mu t + T} + P\,(0 \leq t < 7) \tag{4}$$

where T is a constant and P is a correction that should be determined experimentally. Eq. (4) is the ascending procedure of the nutrient supplementation. To control the cells living in the unbudded period and consisting of rich glycogen, the supplementation rate should decrease after the 7th h. The complete supplementation in yeast cells production can be obtained below:

$$V_t = \{ A\, e^{\mu t + T} + P\,(0 \leq t < 7)\, B\, e^{-(\mu t + T)} + Q\,(7 \leq t < 11) \tag{5}$$

where A (V/h) is the initial supplementation rate and B is the maximum supplementation rate in the 7th h and Q is also a correction. This is the logarithmic feed (*see* Fig. 1), which had been introduced with the hypotheses: The sugar conversion rate and the specific growth rate are considered as averages. Monod (*28*) has demonstrated that the specific cell growth rate in exponential reproduction is constant. The varied specific growth rate observed by Xu et al. (*8*) is owing to the fact that the inoculated cells were always in the G1 phase, followed to a certain extent by synchronous division of the mother cells. The specific growth rate reached maximum in the culture in about 4 h. This is not equivalent to the best nutrient consumption rate of the biomass at later times. The excessive sugar in the medium could enhance ethanol productivity (Crabtree effect) and decrease the yield of biomass if the nutrient supplementation rate reaches maximum during the 4th h. Cell growth was affected by many factors, such as

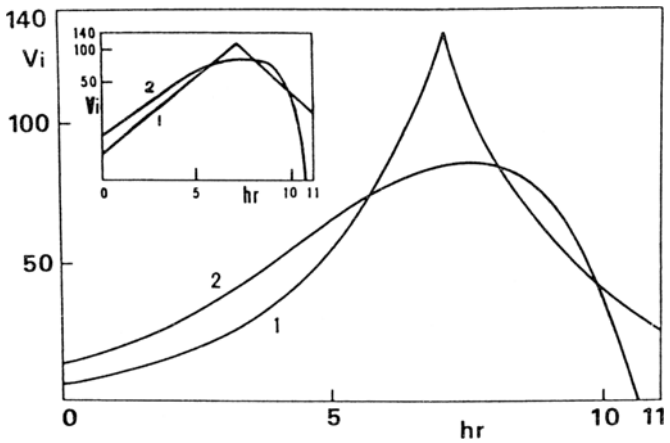

Fig. 1. Parabolic and Exponential Supplementation. Carbon and nitrogen sources were supplemented by parabolic feed, keeping the ethanol concentration in the medium below 0.1% (pH 4.5, 30°C). Curves 1 and 2 respectively represent the logarithmic supplementation rates derived by the instantaneous specific growth rate, the sugar conversion rate, and the parabolic feed simulated by changes in biomacromolecules and dry weight of growing cells derived from the Pasteur effect, as well as the two rates used in the logarithmic feed.

aeration flow, pH, temperature, concentrations of sugar, nitrogen, phosphate, biotine, SO_4^{2-}, Mg^{2+}, Cl^1, and so on. Logarithmic supplementation produced starved cells during the initial stage. However, the nutrient was too abundant at its maximum supplementation rate during the 7th h. This made the yield of yeast appreciably low and wasted nutrient. The logarithmic feed had not rationally dealt with Crabtree effect in the culture.

Parabolic Feed

The consuming carbon source can be considered into two ways, one for reproducible growth and another for Pasteur effect. Therefore, Eq. (6) is derived:

$$V_t = V_i + V_j \tag{6}$$

where V_t represents supplementation rate of the total consumed nutrients; V_i represents the consumption rate of nutrients for reproducible growth; and V_j represents the consumption rate by the Pasteur effect, which is assumed to increase exponentially, because cells reproduce in an exponential fashion:

$$dV_j / dt = \beta V_j \tag{7}$$

where β represents the ethanol production rate. Theoretically, an average β is determined by an average sugar conversion rate (α) (*1,8,27*) and Eq. (8) as follows:

$$V_j = V_o e^{\beta t + U} \quad (8)$$

Where V_o is the initial consumption rate by Pasteur effect and U is a constant that should be determined experimentally. According to Eq. (6),

$$V_i = (A\, e^{\mu t + T}) - (V_o e^{\beta t + U}) + C \quad (9)$$

Here 0.18 was used for μ, because the average specific growth rate (μ) in a 10-h culture was reported as if the biomass reproduced itself five times more than the inoculated (*1–2,8*), and $\beta = 1 - \alpha = 0.433$. The initial sugar consumption rate for the Pasteur effect V_o equals 0.36 mL of 20% molasses (corresponding to 0.072 g sucrose) per g of absolute dry yeast (equivalent to 3.5 g CY) per h, which is 6.26% of A if T and U were assumed to be zero in Eq. (9). C should be −4.42 if 1.0 g dry yeast reproduces itself into 6.0 g in an 11-h culture. When the inoculated cells (equivalent to dry yeast) were n grams and 20% molasses were used,

$$V_i = n\,(5.77e^{\mu t} - 0.36e^{\beta t} - 4.42)\,(t < 11;\ 0 < n < \infty) \quad (10)$$

Integrating Eq. (10), the total consumed sugar was 55.4 mL of 20% molasses (corresponding to 11.72 g sucrose), which was equivalent to that calculated by Xu et al. (*8*), when $n = 1$. Eq. (10), however, should be reformed in the light of the various target products before it is used to culture compressed yeast, active dry yeast, and instant active dry yeast.

Changes of Cells and Biomacromolecules in the Fed-Batch Culture

Parabolic feed has taken the greatest portion of sugar in the logarithmic culture to support the starved cells at the initial supplementation stage. The parabolic feed produced ethanol concentrations in the medium of less than 0.1%. Fig. 2 shows that the budding rate of the inoculated cells is low in the initial stage in which the number of cells is almost a constant. The cells, without division, were in a resting state, but the weight of cells increased during this period. Reproducible growth gave Baratti (*29*). Concentrations of the residual nitrogen and phosphate in the medium were markedly changed during the early stage (data not shown). During this period cells are synthesizing DNA, RNA, proteins, and other compounds. Subsequently, the cell weight increases markedly, conforming to a logarithmic process until the 4th h. Cells increased in a sigmoidal process for the total culture, although cells reproduced synchronously. Eventually, the budding rate leveled at approx 5% of the total cells from the culture.

Fig. 3 shows changes in DNA, RNA, and proteins of cells. Under the parabolic feed, it has been demonstrated that the increase of the biomacromolecules occurs at the initial stage. The synthesis rates of DNA, RNA, and proteins were high until the 8th h, showing sigmoidal curves similar

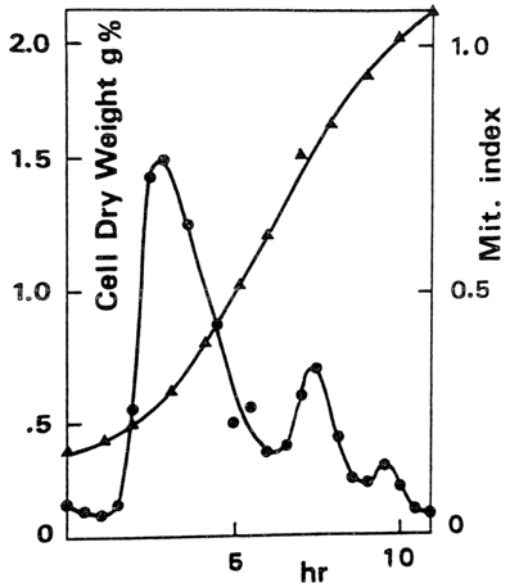

Fig. 2. Changes of Cell Weight and Budding Rate in the Fed-Batch Culture. Fifty mL fermentation broth was taken for centrifugation (4500 rpm, 20 min, 4°C) at the desired time intervals. The cells were dried (4 h, 105°C) and weighed after the temperature was returned to 25°C in a desiccator. The budding rate was determined under a microscope according to Thomas (World Health Organization, 1980). Curves 1 and 2 respectively represent changes in cell weight (g%) and budding rate (%).

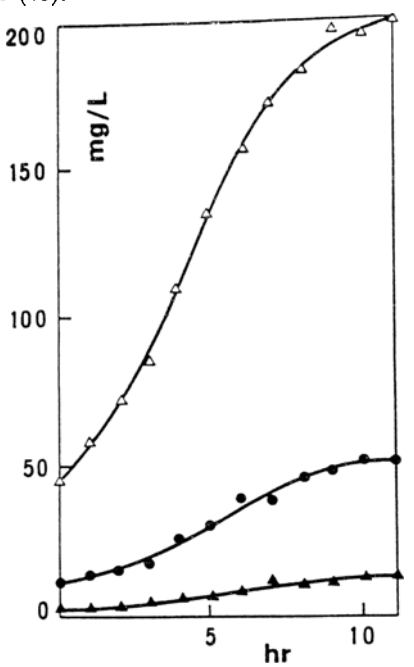

Fig. 3. Changes of Biomacromolecules of Cells in the Fed-Batch Culture. The conditions were as in Fig. 1, except measurement of cells DNA, RNA, and proteins were from the fermentation broth of the fed-batch culture. Curves 1 and 3 respectively represent concentrations of RNA, DNA, and total proteins of the cells (mg/L).

to that of total cell weight. Kinetics of the nutrient supplementation should conform to changes in biomass, DNA, RNA, and proteins as well as producible growth that has been made by the parabolic feed process.

Parabolic Feed for the Other Species

To culture cells producing byproducts, the parabolic nutrient supplementation could be represented as follows:

$$V_i = n\,(A\,e^{\mu t + T} - V_o e^{\beta t + U} + C)\ (0 < n < \infty) \tag{11}$$

The definition of the parameters in Eq. (11) are similar to Eq. (9), except β represented the sugar consumption rate for byproduct production. The parameters should be revised according to the different species of cells employed. The prerequisites to use this equation are that the byproducts can better inhibit the producible growth after their concentration is improved in the media. The quantity of the inoculated cells should be large (e.g., Baker's yeast was 1.0% of medium g/V) and the time for a fed-batch culture should be short (e.g., 11 or 14 h for Baker's yeast).

DISCUSSION

Although production of Baker's yeast has been carried out for hundreds of years, there have been described many different techniques for Baker's yeast production, especially the methods of supplementation of carbon, nitrogen, and the other nutrient sources. A few scientists and engineers have reported on methods of nutrient supplementation for the fed-batch cultures (*10*). The logarithmic feed and the supplementation controlled by the ethanol concentration have been used in production of Baker's yeast (*3,7–8,10*). Different methods can produce different qualitative and quantitative yeast, depending on the different target products.

Logarithmic supplementation derived by changes in the number of cells requires a hypothesis that the specific growth rate and sugar consumption rate are constants. Moreover, it was reported that the specific growth rate was a constant in the exponential division of cells (*28*). However, logarithmic supplementation does not deal rationally with the Crabtree effect, which definitely affected the growth of cells. It did not distinguish the young cell and the mother cell but counted them as two cells. These cells metabolize such that they have different sugar consumption rates. This is the reason that the logarithmic feed requires modification.

In the final amplification, the cells reproduce themselves synchronously. Changes of DNA, RNA, and proteins, however, follow a sigmoidal process similar to that of cell mass (3). At the same time, Pasteur and Crabtree effects, which can be eliminated by the appropriate supplementation of carbon source and aeration flow, markedly decrease the yield of

Table 1
Specifications of ADY Made by the Parabolic and the Exponential Supplementation Process in an 11-h Fermentation[a]

Qualifications	Parabolic	Exponential[a]
Lag time	About 2 h	About 2.5 h
Sugar conversion rate	About 50%	45–50%
Proliferation in 11 h course	About 6 times	About 5 times
GPR of product[a]	>750 mL	>750 mL
Shelf life[b]	>10 mo	>10 mo
Trehalose quantity[c]	14–16%	12–14%
Nitrogen content[d]	About 7%	About 7%
Budded cells[e]	<5%	<5%

[a]For the qualifications of the product by the exponential method refer to Xu et al. (*8*)
[b]demonstrated with Felsher (*9*)
[c]Ballentine (*22*) and Markham (*23*)
[d]determined by Trevelyan and Harrison (*26*)
[e]calculated by the method of Thomas (*16*).

cells. In general, to inhibit the effects and improve yield of the yeast, the best method is the parabolic feed method, because it has been designed to account for changes in growth, and biomacromolecule synthesis, derived from the specific growth rate, the sugar conversion rate, and the Pasteur effect. It will make the culture of yeast rational and guaranteed, saving thousands of tons of sugar, and producing millions of tons of active biomass.

The phase of the cell life cycle is important to the quality of the biomass, such as GPR, storage life, conversion rate of ADY, and so on. ADY would be impossible to be made if the cells were in random phases of their cycles. They would be subjected to serious dehydration caused by moisture loss from 70% to about 9% during conversion into ADY. The budded cells are not able to survive a 4-h dehydrating procedure because cells in different life cycle phases have different features. Descending the nutrient supplementation rate after the 7th h (logarithmic feed) or 8th h (parabolic feed) has been derived to bring yeast to the G1 phase, accompanied by glycogen accumulated in the plasma. The glycogen could be converted into trehalose that supports energy to cells for their survival in the dehydration heating process. Cells in the G1 are more resistant to drying and aging, features particularly important in the process of producing dry yeast. Low mol wt of RNA has a marked effect of serious dehydration. It suggests that metabolism of DNA, RNA, and proteins may change and adapt to the dehydration process (*30*). It is impossible in a continuous culture to bring all the cells into G1 phase at the same time. It is difficult to produce ADY from a biomass model on a continuous basis.

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