Journal of Industrial Microbiology (1996) 16, 117–123

Growth kinetics of *Saccharomyces cerevisiae* in batch and fed-batch cultivation using sugarcane molasses and glucose syrup from cassava starch

SS Win[1], A Impoolsup[2] and A Noomhorm[1]

[1]*School of Environment, Resources and Development, Asian Institute of Technology, Bangkok 10501;* [2]*Department of Biotechnology, Mahidol University, Bangkok 10400, Thailand*

Growth kinetics of *Saccharomyces cerevisiae* in glucose syrup from cassava starch and sugarcane molasses were studied using batch and fed-batch cultivation. The optimum temperature and pH required for growth were 30°C and pH 5.5, respectively. In batch culture the productivity and overall cell yield were 0.31 g L^{-1} h^{-1} and 0.23 g cells g^{-1} sugar, respectively, on glucose syrup and 0.22 g L^{-1} h^{-1} and 0.18 g cells g^{-1} sugar, respectively, on molasses. In fed-batch cultivation, a productivity of 3.12 g L^{-1} h^{-1} and an overall cell yield of 0.52 g cells g^{-1} sugar were achieved in glucose syrup cultivation and a productivity of 2.33 g L^{-1} h^{-1} and an overall cell yield of 0.46 g cells g^{-1} sugar were achieved in molasses cultivation by controlling the reducing sugar concentration at its optimum level obtained from the fermentation model. By using an on-line ethanol sensor combined with a porous Teflon® tubing method in automating the feeding of substrate in the fed-batch culture, a productivity of 2.15 g L^{-1} h^{-1} with a yield of 0.47 g cells g^{-1} sugar was achieved using glucose syrup as substrate when ethanol concentration was kept at a constant level by automatic control.

Keywords: baker's yeast; *Saccharomyces cerevisiae*; fed-batch cultivation; ethanol sensor

Introduction

Sugar cane molasses is commonly used as a raw material in the manufacture of baker's yeast. However, there are some problems associated with the process, such as the dark color of the product and an unpredictable variation in quality. Alternately, cassava is a high energy carbon source. Even though the glucose syrup from cassava starch might be more expensive than molasses there are advantages in terms of product quality and ease of waste water treatment. Thailand is one of the biggest producers and exporters of cassava in the world and produced about 18 million tons in 1993. A comparison has therefore been made between glucose syrup from cassava starch and sugarcane molasses as carbon and energy sources for *Saccharomyces cerevisiae*.

In batch yeast cultivation, high sugar concentration in the culture can result in Crabtree repression which inhibits respiratory enzymes and increases ethanol production. This problem can be overcome by fed-batch cultivation where essential nutrients can be fed incrementally to the bioreactor during cultivation. To optimize the cultivation process in order to get maximum cellular yield and high productivity, a suitable control strategy has to be developed [5]. Many on-line sensors have been applied to on-line measurements and control of process variables: for example, O_2 gas analyzers and infrared CO_2 analyzers have been used to control sugar feed so as to maintain the respiratory quotient, RQ, within the range of 1.0 to 1.1 throughout cultivation [2]. However, the absence of suitable detection methods and the high cost of equipment limit the use of on-line sensors.

On-line measurement of ethanol concentration in the culture broth during cultivation can also be employed as a parameter for the control system because ethanol production is the major reason for the lowering of the maximum cellular yield. The ethanol concentration in the culture broth can be monitored by using a porous Teflon® tubing method in which ethanol is separated from the culture broth and is analyzed by alcohol sensor. This porous Teflon® tubing is permeable to volatile substrates such as ethanol and acetic acid. Air flowing along the tube at a low flow rate is used to carry ethanol which diffuses through the membrane wall of the tubing. Ethanol carried by the air is analyzed by allowing it to vaporize inside the ethanol sensor [10].

The objectives of this study were to: (1) compare the productivities and overall cell yields by using two substrates: sugarcane molasses and glucose syrup from cassava starch in batch and fed-batch cultivations; (2) determine feeding procedure for optimum productivity and overall cell yield by controlling the reducing sugar concentration at optimum level in fed-batch cultures using both substrates; and (3) test an automatic control of a fed-batch bioreactor by keeping ethanol concentration at a constantly low level by using an on-line ethanol sensor.

Materials and methods

Microorganism

Saccharomyces cerevisiae (TISTR 5020 W Daengsubha, active dry yeast) was obtained from the Thailand Institute

Correspondence: A Noomhorm, School of Environment, Resources and Development, Asian Institute of Technology, GPO Box 2754 Bangkok 10501, Thailand
Received 22 December 1994; accepted 22 November 1995

of Science and Technological Research (TISTR), Bangkok, Thailand. The culture was maintained by sub-culturing it on yeast malt extract agar and storage at 4°C.

Fermentation substrates

Two types of substrates, namely, sugar cane molasses (Wang Kanai Sugar Industry Co, Suphanburi Province) and glucose syrup from cassava starch (Rangsit Co, Pathumtani Province) were used as the sole carbon and energy source. Sugar cane molasses was pretreated by acidifying it with sulfuric acid (95%) to pH 4 and heating it to 120°C for 1 min in order to precipitate some inorganic materials and suspended matter. It was then centrifuged at 2327 × *g* for 5 min to remove the precipitate.

Media

Agar slants for sub-culture were prepared by dissolving 3 g of yeast extract, 3.0 g of malt extract, 5.0 g of peptone, 10.0 g of glucose and 20.0 g of agar in 1 L distilled water. Growth medium (1 L) contained: $(NH_4)_2SO_4$ 10 g, KH_2PO_4 6 g, $MgSO_4{\cdot}H_2O$ 3 g, $CaCl_2{\cdot}2H_2O$ 0.1 g, NaCl 0.1 g and vitamin solution 10 ml. The vitamin solution contained (per L): thiamine 30.0 mg, biotin 0.5 mg, *m*-inositol 30.0 mg, pyridoxine 0.5 mg and Ca-pantothenate 1.0 mg. The vitamin solution was filter-sterilized using a Millipore filter (Goretex Inc, Okayama, Japan) (0.45-μm pore size) [9]. The sugar concentration used for batch cultivation had 5% reducing sugar and that for fed-batch growth medium had 20% reducing sugar.

Inoculum preparation

The inoculum for the bioreactor was obtained by cultivation in two stages. In the first stage, two 500-ml Erlenmeyer flasks, each containing 50 ml of sterilized growth medium, were inoculated from slants of *S. cerevisiae* and incubated for 20 h at 30°C and 120 rpm. Two 1000-ml Erlenmeyer flasks each containing 250 ml sterilized growth medium were then inoculated with the contents from the first stage and incubated for 20 h at 30°C and 120 rpm in a rotary incubator.

Experiments using shake flasks

Two environmental conditions, temperature and pH were tested in order to determine the values which would support maximum growth of *S. cerevisiae*. A series of 500-ml Erlenmeyer flasks containing 125 ml of medium with either glucose syrup or molasses as the carbon source were set up at 5% reducing sugar concentration. To determine the optimum temperature, the medium in each flask was adjusted to pH 4.5 with 0.1 N HCl and 1 N NaOH. Ten milliliters of the inoculum of 0.65 optical density (610 nm) was introduced into each flask and incubated for 24 h at 120 rpm at temperatures of 20°C, 25°C, 30°C, 35°C and 40°C, respectively.

To determine the optimum pH, the same procedure was followed in setting up the flasks. However the medium was adjusted to pH 4.0, 4.5, 5.0, 5.5, or 6.0. The flasks were incubated at 120 rpm and at the optimum temperature obtained from the temperature test.

Growth studies using a bioreactor

Cultivation was carried out in a stirred tank reactor (Applikon, BV Schiedam, Holland) with a working volume of 12 L. For batch cultivation, the temperature was kept at 30 ± 1°C and the pH of the medium was controlled at pH 5.5 ± 0.2 by the automatic addition of 1 N HCl and 1 N NaOH. The agitator speed and air flow rate used in batch cultivation were 500 rpm and 2.5 L min^{-1}, respectively. Samples were taken to measure the concentrations of cells, glucose and ethanol. They were centrifuged at 1782 × *g* for 5 min and the cell-free supernatant fluid was stored at 10°C. The ethanol concentrations were determined by gas chromatography.

The productivity and overall cell yield were described as follows:

$$\text{Productivity} = \frac{\text{maximum cell concentration} - \text{initial cell concentration}}{\text{total time of cultivation}} \quad (1)$$

$$\text{Overall cell yield} = \frac{\text{total biomass formed}}{\text{total sugar used}} \quad (2)$$

For fed-batch cultivation, the yeast cells were transferred from the shake flask to the bioreactor and grown for 1 h as a batch culture before the commencement of feeding. The rotation speed of the impeller and the air flow rate were kept at 500 rpm and 2.5 L min^{-1}, respectively, at the beginning of cultivation. During cultivation dissolved oxygen was maintained above 15% to avoid oxygen limitation. Feed addition was accomplished with a peristaltic pump.

The strategy underlying the operation of fed-batch cultivation is based on matching the supply of carbon with demand [3,9]. The feed rate of nutrients was a function of total yeast dry cell mass, and specific growth rate. Assuming a complete mixing of the medium in a well-agitated and aerated bioreactor, the feed rate (F_i) for i^{th} interval can be assessed for a quasi-steady state and specific case of $S_R >> S_i$:

$$F_i = \frac{\mu_i X_i V_i}{Y_i S_R} \quad (3)$$

where μ is specific growth rate, X_i is the total amount of cells in the bioreactor, Y is the yield coefficient, V_i is the calculated broth volume, S_R is the reducing sugar concentration of the feed and S_i is the initial reducing sugar concentration in the bioreactor. The feed rate was based on estimated specific growth rate and calculated volume.

Growth model

Assuming the yield as constant throughout the cultivation period, the growth models and associated parameters were determined by developing a small basic program for both substrates.

The growth model which was adopted in this study was a substrate inhibition model and is:

$$\mu = \frac{\mu_m S}{K_s + S + S^2/K_i} \quad (4)$$

where μ_m = maximum specific growth rate (h^{-1}), μ = spec-

ific growth rate (h^{-1}), S = substrate concentration ($g\ L^{-1}$), K_s = saturation constant ($g\ L^{-1}$), K_i = inhibition constant ($g\ L^{-1}$).

The parameters from the growth model and the optimum levels of reducing sugar were determined from non-linear regression between specific growth rate and the concentration of reducing sugar.

Feed rate for controlling reducing sugar concentration at optimum level

Agrawal and co-workers [1] used an algorithm implemented by an on-line estimate of specific fermentation rate without measurement of nutrient concentration. In this study, a feeding procedure (Figure 1) was developed by combining estimates of specific growth rate and the concentration of reducing sugar. From previous fed-batch cultivations, the optimum reducing sugar concentration and corresponding optimum specific growth rate of the yeast in both substrates were determined. Based on this, the reducing sugar concentration was assigned to one of three conditions: below optimum range (S_{LOPT}), optimum range (S_{OPT}), or above optimum range (S_{HOPT}). The specific growth rate used for time zero was 25% higher than μ_{OPT} to avoid substrate limitation.

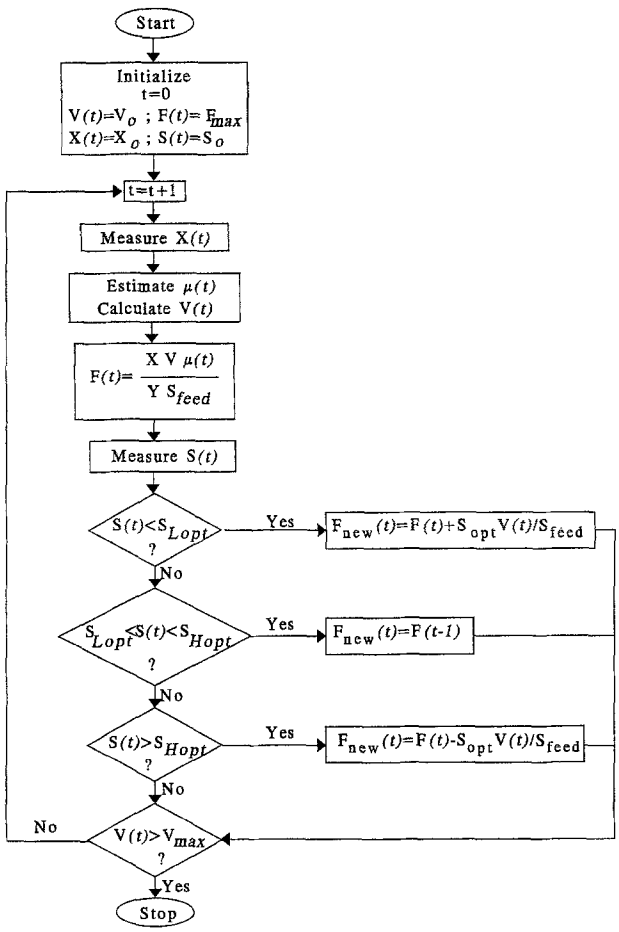

Figure 1 Feeding procedure of substrate to control the reducing sugar concentration at optimum level.

Ethanol on-line control in fed-batch cultivation

The alcohol sensor (Nippon Ceramic Co, NGSX-03, Nagoya, Japan) was used in this study for on-line measurement of ethanol in the cultivation broth. The ethanol sensor was connected to Teflon® tubing which could be immersed in the cultivation broth medium. The dimensions of the tubing were 2.0 mm × 0.4 mm × 1000 mm (inside dia. × thickness × length). Air at a flow rate of 40 ml min^{-1} was forced through the tube to carry to the sensor ethanol vapour which had diffused through the wall of teflon tube. The electric signal (mV) thus produced was monitored using a multimeter. Before using the sensor, a calibration curve was developed between concentration and mV.

The set point of ethanol concentration was 200 ppm. The control system usually started after manual operation for 10–30 min when the concentration of ethanol reached the desired value [4]. A peristaltic pump (Cole Palmer Instrument Co, Chicago, IL, USA) was used for feeding medium.

Analytical methods

Optical densities measured at 610 nm were converted to dry cell mass concentration after establishing a calibration chart. The modified dinitrosalicylic acid [6] and Somogyi-Nelson methods [8] were used for determination of reducing sugar in the broth media containing glucose syrup and sugar cane molasses. Ethanol concentration was analyzed using a gas chromatograph equipped with a flame ionization detector.

Results

Shake flasks

Figure 2 shows the changes in growth, expressed as dry weight of *S. cerevisiae* after 24 h of incubation over a temperature range of 20°C to 40°C. Growth on both substrates was significantly affected by the incubation temperature. The optimum temperature for the growth of *S. cerevisiae*

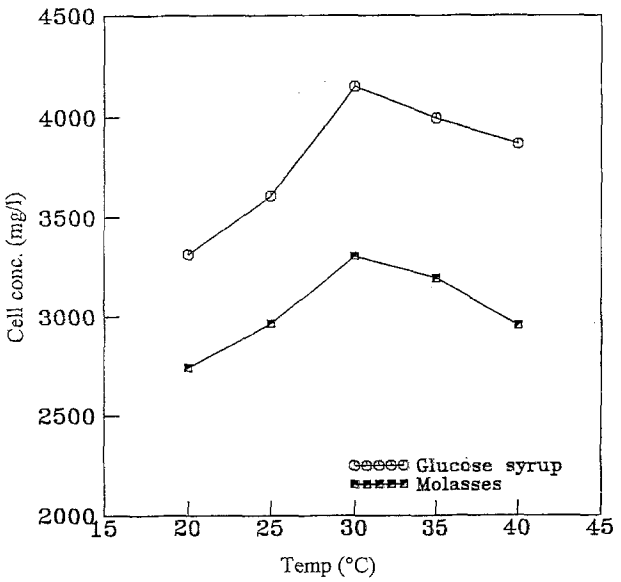

Figure 2 Effect of temperature on cell growth (pH = 4.5) using glucose syrup or molasses as substrates.

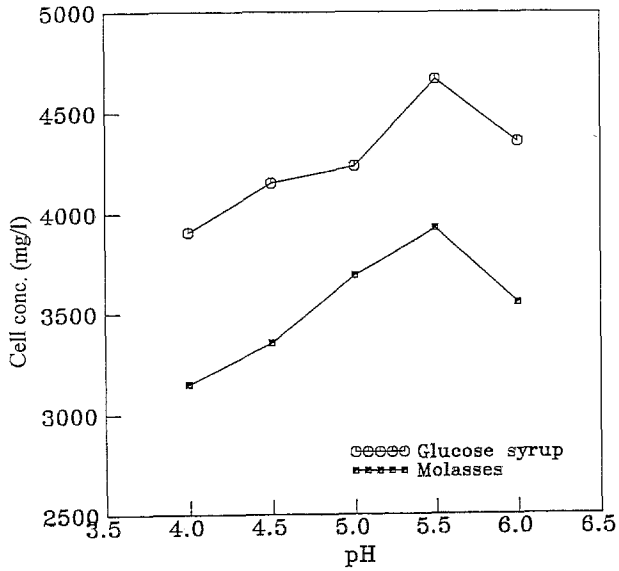

Figure 3 Effect of pH on cell growth (T = 30°C) using glucose syrup or molasses as substrates.

in both glucose syrup and molasses was 30°C. *S. cerevisiae* grew best at pH 5.5 on either substrate (Figure 3).

Batch cultivation

Figures 4 and 5 show the changes in the concentration of dry cell mass, reducing sugar and ethanol produced during batch cultivation. Molasses gave a lower maximum cell concentration and greater ethanol production than glucose syrup. The composition of molasses is complicated and because of invertase activity, sucrose can be hydrolyzed to glucose and fructose during cultivation resulting in a high sugar concentration. With glucose syrup, a productivity of 0.31 g L^{-1} h^{-1} with an overall cell yield of 0.23 g cells g^{-1} sugar were obtained. A productivity of 0.22 g L^{-1} h^{-1} and an overall cell yield of 0.18 g cells g^{-1} sugar were found with molasses medium. These were lower than those obtained using glucose syrup.

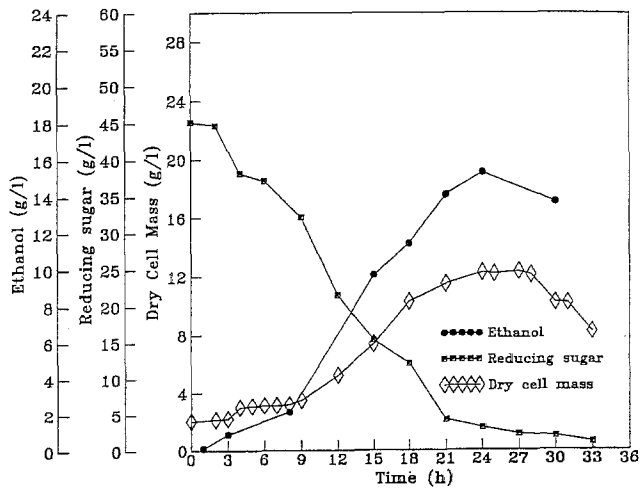

Figure 4 Relationship between cell, reducing sugar, and ethanol concentrations vs time in batch cultivation using glucose syrup as substrate.

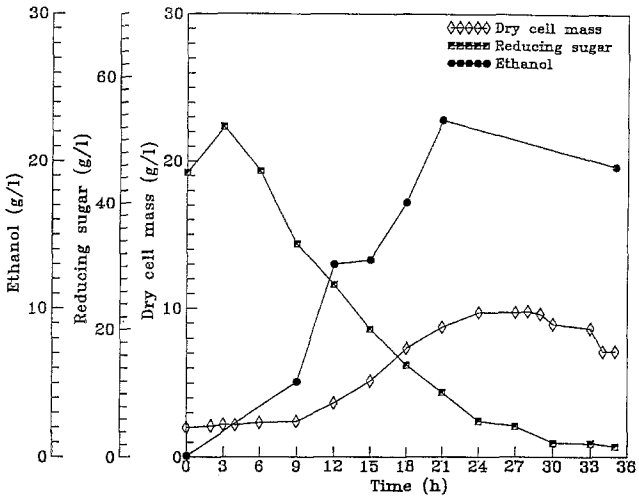

Figure 5 Relationship between cell, reducing sugar, and ethanol concentrations vs time in batch cultivation using sugarcane molasses as substrate.

Fed-batch cultures

Figure 6 presents the relationship between dry cell mass, reducing sugar and ethanol concentration vs time on glucose syrup. Not much difference in ethanol production was found during the first 6 h while the reducing sugar concentration was below 0.35 g L^{-1}. When the glucose concentration increased above 0.8 g L^{-1}, the ethanol concentration showed its maximum value of 1.35 g L^{-1}. The specific growth rate decreased during cultivation (Figure 7). A final cell concentration of 45.19 g L^{-1} with an overall cell yield of 0.5 g cells g^{-1} sugar and productivity of 2.52 g L^{-1} h^{-1} were obtained.

Figure 8 presents the relationship between dry cell mass, reducing sugar and ethanol concentration with time for the fed-batch cultivation using molasses. The accumulation of reducing sugar was higher than that in the glucose syrup fed-batch culture. Figure 9 presents the changes in specific growth rate during the cultivation. For the first 3 h, higher

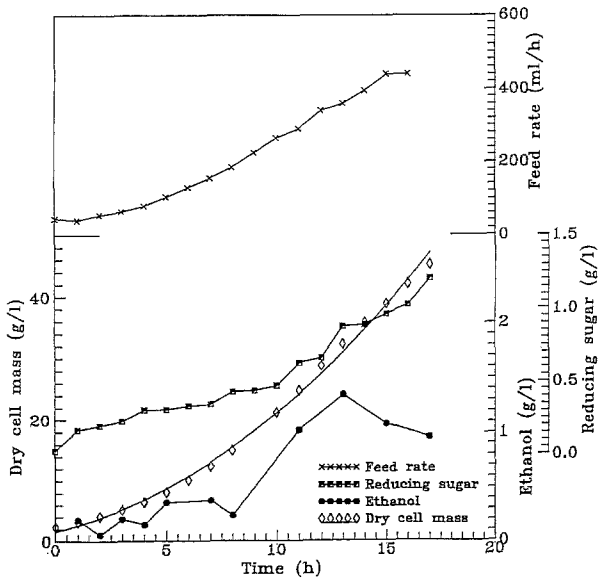

Figure 6 Cell, reducing sugar, ethanol concentrations and feed rate vs time in fed-batch cultivation using glucose syrup.

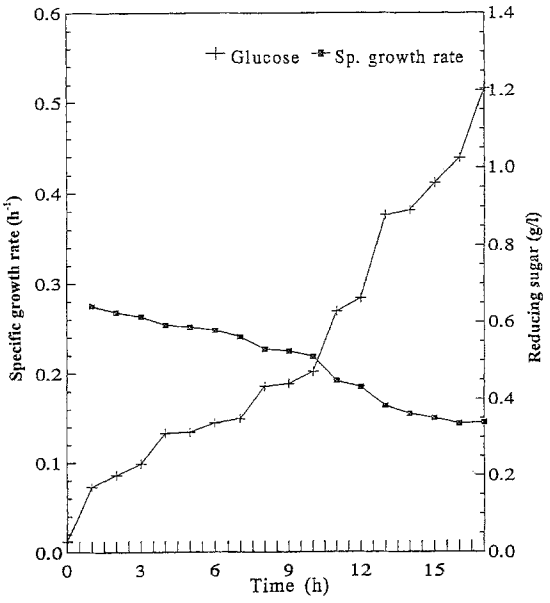

Figure 7 Glucose concentration and specific growth rate in fed-batch cultivation using glucose syrup.

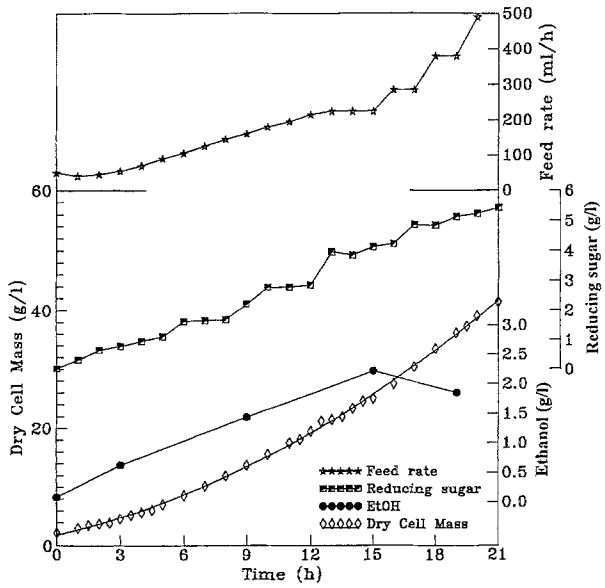

Figure 8 Cell yield, reducing sugar and ethanol concentrations and feed rate vs time in fed-batch cultivation using sugarcane molasses.

specific growth rates were found but they decreased below 0.15 h^{-1} after 4 h. DO was manually controlled so as to maintain > 15% saturation. The final cell concentration obtained from this experiment was 41.36 g L^{-1} and the overall cell yield was 0.41 g cells g^{-1} sugar with a productivity of 1.86 g L^{-1} h^{-1}.

Ethanol production was lower and cell concentration was higher in the fed-batch cultivation compared with batch cultivation. Thus, the productivity and the overall cell yield of fed-batch cultivation are very much higher than those of batch culture. In batch cultures an excess of sugar would lead to anaerobic growth resulting from lack of oxygen and lead to ethanol production instead of yeast biomass production [7], which would result in low productivity and low overall cell yield.

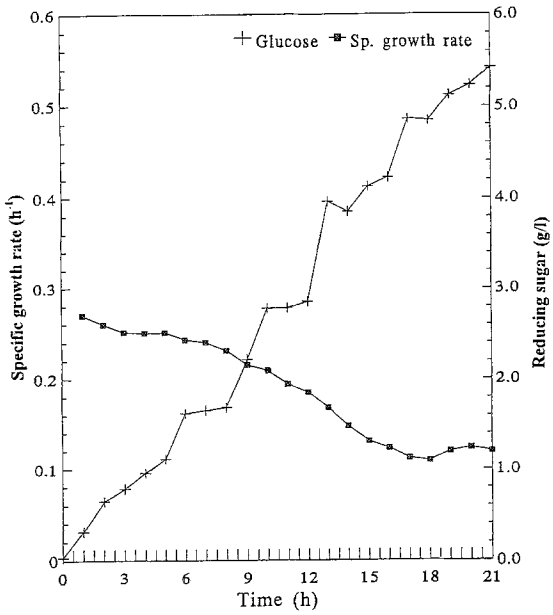

Figure 9 Glucose concentration and specific growth rate in fed-batch cultivation using sugarcane molasses.

Fed-batch cultivation at optimum reducing sugar concentration

The substrate inhibition model (Equation 4) was adopted and associated parameters from the growth model which were determined from the non-linear regression are presented in Table 1. Yeast cells showed their optimum specific growth rates at glucose concentrations of 0.14–0.17 g L^{-1} in the fed-batch cultivation using glucose syrup, while they showed their optimum specific growth rates at reducing sugar concentration of 0.5–0.7 g L^{-1} in the fed-batch culture using molasses as substrate.

The change in feed rate with respect to dry cell mass, glucose concentration, and calculated volume of the broth medium inside the reactor during glucose syrup cultivation is shown in Figure 10. The figure also presents the relationship between dry cell mass, glucose, and ethanol concentrations with time. At the end of cultivation, reducing sugar and ethanol concentrations were 0.11 g L^{-1} and 0.48 g L^{-1} respectively. Biomass increased throughout the process and reached 49.21 g L^{-1} at the end of the cultivation. The productivity of 3.12 g L^{-1} h^{-1} and overall cell yield of 0.52 g cells g^{-1} sugar were higher than the previous fed-batch cultivation on glucose syrup.

Figure 11 presents the changes in concentrations of cells, reducing sugar, and ethanol with time according to feed rates calculated from feed procedure 1 during cultivation on molasses. The reducing sugar concentration was maintained at the optimum range of 0.5–0.7 g L^{-1} throughout

Table 1 Associated parameters of fermentation models for fed-batch cultures

Substrate	μ_{max} (h^{-1})	K_s (g L^{-1})	K_i (g L^{-1})
Glucose syrup	0.4	0.05	0.55
Molasses	0.5	0.27	1.54

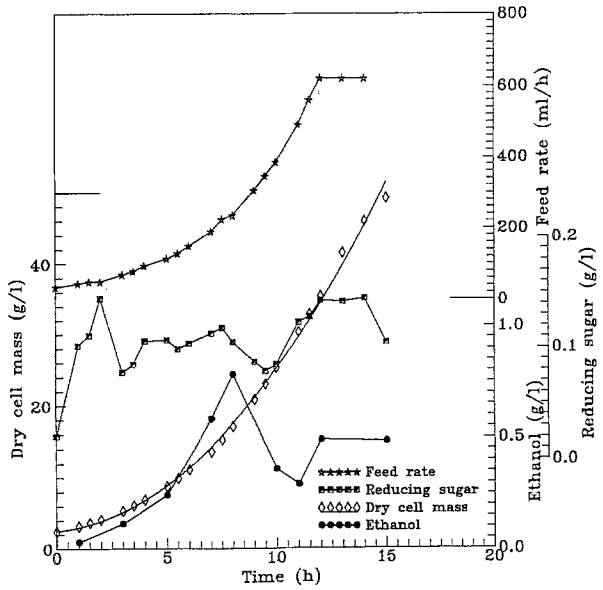

Figure 10 Dry cell mass, reducing sugar, ethanol concentrations and feed rate vs time in fed-batch cultivation feeding glucose syrup to achieve an optimum reducing sugar level.

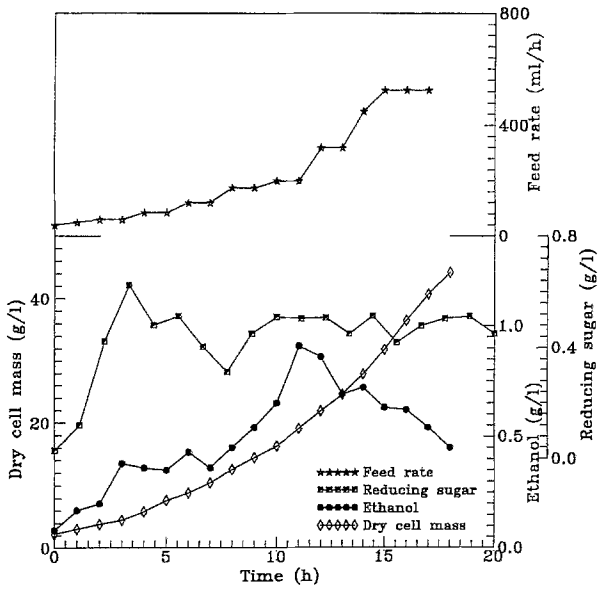

Figure 11 Dry cell mass, reducing sugar, ethanol concentrations and feed rate vs time in fed-batch cultivation feeding molasses to achieve an optimum reducing sugar level.

the cultivation. The ethanol concentration was found to increase after 3 h of cultivation but it was lower than that in the cultures where the reducing sugar concentrations were not controlled. In the last hour of cultivation, the reducing sugar concentration was at an optimum with decreasing ethanol concentration. At the end of cultivation, ethanol concentration was 0.45 g L^{-1} and the biomass reached 44.2 g L^{-1}. The productivity of 2.33 g L^{-1} h^{-1} and overall cell yield of 0.46 g cells g^{-1} sugar were higher than the previous fed-batch cultivation on molasses.

The experimental data show that higher productivity and overall cell yield with high specific growth rate could be achieved in glucose syrup than in molasses by maintaining reducing sugar at its optimum level. By using feeding procedure 1, the feed rate calculated from Equation 3 was corrected by analyzing the reducing sugar concentration existing at the sampling time and excess reducing sugar could be reduced to maintain an optimum level resulting in higher productivity and cell yield.

Fed-batch cultivation with application of ethanol sensor coupled with Teflon® tubing

To test the ethanol sensor by using Teflon® tubing for on-line measurement of ethanol in the fed-batch cultivation broth using syrup, the ethanol concentration was maintained at 200 ppm. After 7 h of cultivation, total volume reached 6 L, and a productivity of 2.15 g L^{-1} h^{-1} and overall cell yield of 0.47 g cells g^{-1} sugar were achieved (Figure 12). During cultivation, the reducing sugar concentration decreased from 0.9 to 0.4 g L^{-1} and the ethanol concentration fluctuated less from the set point.

Discussion

These experiments were conducted to study the growth kinetics of *S. cerevisiae* on two different substrates, glucose syrup and sugarcane molasses, under different cultivation procedures. The cultivation procedures tested were batch, fed-batch manual control and fed-batch automatic control. The ethanol concentration was used as the parameter in an automatic control procedure. An on-line ethanol sensor was used to automate feeding of substrate. The optimum temperature required for the growth of *S. cerevisiae* was 30°C and the optimum pH required was 5.5, irrespective of the substrate used.

In batch culture, the productivity and overall yield on glucose syrup were higher than those obtained on molasses. The productivity and overall cell yield on glucose syrup were 0.31 g L^{-1} h^{-1} and 0.23 g cells g^{-1} sugar respectively, and on molasses were 0.22 g L^{-1} h^{-1} and 0.18 g cells g^{-1} sugar, respectively.

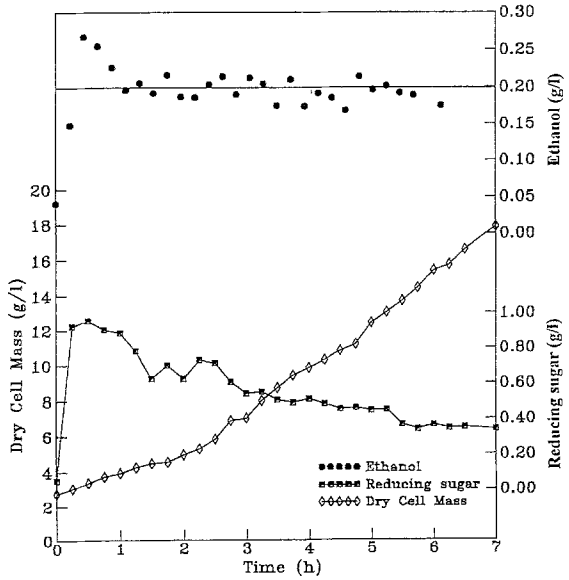

Figure 12 Dry cell mass, reducing sugar, and ethanol concentrations vs time in fed-batch cultivation using ethanol sensor for on-line measurement.

In fed-batch culture, glucose syrup cultivation gave a higher productivity and overall cell yield than those of molasses cultivation. Productivity of 2.52 g L^{-1} h^{-1} and an overall cell yield of 0.5 g g^{-1} sugar were achieved in glucose syrup cultivation whereas molasses gave a productivity of 1.86 g L^{-1} h^{-1} and cell yield of 0.41 g cells g^{-1} sugar. By controlling the reducing sugar concentration at its optimum level, higher productivity and overall cell yields were achieved irrespective of the substrate used. A productivity of 3.12 g L^{-1} h^{-1} and overall cell yield of 0.52 g cells g^{-1} sugar were obtained with glucose syrup, and 2.33 g L^{-1} h^{-1} and 0.45 g cells g^{-1} sugar were obtained with molasses. Glucose syrup from cassava starch gave higher productivity and overall yield of cells than sugarcane molasses in both batch and fed-batch cultivations. The productivity and overall yield of cells were increased by controlling reducing sugar concentration at an optimum level which was determined from the growth model.

A simple on-line ethanol sensor combined with Teflon® tubing can be successfully used in fed-batch cultivation to control ethanol concentration. The ethanol concentration in the cultivation broth was automatically controlled without the use of gas chromatography and a productivity of 2.15 g L^{-1} h^{-1} and overall cell yield of 0.47 g cells g^{-1} sugar was achieved using glucose syrup as substrate. Therefore, computer-aided operation of fed-batch fermentation using a simple and economical on-line ethanol sensor combined with Teflon® tubing would be useful for the industrial production of baker's yeast.

Acknowledgements

The authors are grateful to Japanese Goretex Inc, Okayama, Japan for providing the Teflon® tubing for this research.

References

1 Agrawal P, G Koshy and M Ramseier. 1989. An algorithm for operating a fed-batch fermenter at optimum specific-growth rate. Biotechnol Bioeng 33: 115–125.
2 Aiba S, S Nagai and Y Nishizawa. 1976. Fed-batch culture of *Saccharomyces cerevisiae*: a perspective of computer control to enhance the productivity in baker's yeast cultivation. Biotechnol Bioeng 18: 1001–1016.
3 Cooney CL, HY Wang and DIC Wang. 1977. Computer-aided material balancing for production of fermentation parameters. Biotechnol Bioeng 19: 55–67.
4 Dairaku K and T Yamane. 1979. Use of porous teflon tubing method to measure gaseous or volatile substances dissolved in fermentation liquids. Biotechnol Bioeng 21: 1671–1676.
5 Fatimah AB, A Arbakariya and AB Fadzilla. 1992. Growth kinetics study of bakers' yeast (*Saccharomyces cerevisiae*). ASEAN Food J 7: 205–206.
6 Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31: 426–428.
7 Reed G. 1982. Production of baker's yeast. In: Prescott and Dunn's Industrial Microbiology. 4th edn, pp 593–633, AVI Publishing Co, Westport, CT.
8 Somogyi M. 1945. A new reagent for the determination of sugars. J Biol Chem 61: 160.
9 Wang HY, CL Cooney and DIC Wang. 1979. Computer control of baker's yeast production. Biotechnol Bioeng 21: 975.
10 Yamane T, M Matsuda and E Sada. 1981. Application of porous teflon tubing method to automatic fed-batch culture of micro-organisms. I. Automatic constant-value control of fed substrate (ethanol) concentration in semibatch culture of yeast. Biotechnol Bioeng 23: 2509–2524.