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Enhancement of the Efficiency of Bioethanol Production by *Saccharomyces cerevisiae* via Gradually Batch-Wise and Fed-Batch Increasing the Glucose Concentration

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Abstract: High initial glucose concentrations may inhibit glucose utilization and decrease ethanol fermentation efficiency. To minimize substrate inhibition, the effects of feeding yeast with different glucose concentrations on the ethanol production by batch and fed-batch cultures in a 5-L fermentor were investigated. When a batch culture system with *Saccharomyces cerevisiae* was used for ethanol fermentation with glucose concentrations ranging 10–260 g/L, as a result, 0.2–7.0 g/L biomass and 5.1–115.0 g/L ethanol were obtained. However, substrate inhibition was observed with the initial glucose concentrations greater than 200 g/L in the fermentative media. When a fed-batch culture system (an initial glucose concentration of 180 g/L and total glucose concentration of 260 g/L) was performed, the maximum ethanol concentrations and ethanol yield were significantly higher than those of the batch cultures. The cell biomass, maximum ethanol concentration, and ethanol yields for the fed-batch fermentation cultures were 8.3 g/L, 130.1 g/L and 51% (100% of the theoretical value), respectively. The results indicated that high ethanol concentration and ethanol yield could be achieved by the fed-batch cultures with total glucose concentrations up to 260 g/L.

Keywords: bioethanol; glucose inhibition; batch culture; fed-batch culture; fermentor

1. Introduction

The increasing consumption of crude oil and greenhouse effects connected with petroleum combustion has initiated a worldwide concern for the development of alternative and renewable fuels that are environmentally friendly. Bioethanol is believed to be one such alternative, which can be produced from renewable biomasses such as sugar, starch and cellulosic materials. Bioethanol is also a renewable energy source that is well positioned to be an excellent clean-burning, high-energy alternative fuel to gasoline. Nowadays, bioethanol is commonly blended with conventional gasoline for use in automobile fuels to reduce greenhouse gases emissions. Because the raw materials used to produce bioethanol come from renewable resources with low CO_2 emissions, bioethanol has offered many distinctive advantages over fossil fuels [1].

Saccharomyces cerevisiae is the well-known brewing yeast that can ferment glucose into ethanol under anaerobic conditions. The microorganism is ideal for ethanol production because it possesses

several properties, including: fast growth rates, efficient glucose utilization and ethanol production, and a tolerance for environmental stresses such as high ethanol concentration [2], wide range of pH [3] and low oxygen levels [4]. Similar to in most organisms, the metabolic pathway of glycolysis converts glucose into pyruvate, which can then be fermented into ethanol under anaerobic conditions. The energy for growth of *S. cerevisiae* cells during ethanol fermentation is mostly provided by the glycolytic pathways. Further, ethanol production by *S. cerevisiae* is economically efficient and industrially feasible because *S. cerevisiae* reproduces quickly and thus meets the requirements of the large pilot-scale processes [5]. The growth of a microorganism is strongly influenced by medium composition, and the production of ethanol is cell-dependent. The rapid growth of *S. cerevisiae* in batch cultures with limited nutrients results in the production of ethanol; however, high initial glucose concentration in the fermentative media may cause substrate inhibition, which substantially lowers the fermentation efficiency. Thus, for an effective fermentation process, the major concern is the optimization of the composition of cultural media and parameters. To meet industry demand, it is also necessary to improve the performance of the system and increase the ethanol yield without increasing the cost of production [6,7]. Besides, if ethanol fermentation is to be of practical use in the industry, the ethanol yield can constitute as much as 93% of its theoretical value of the ratio of ethanol produced to sugar consumed [8].

High initial glucose concentrations in the culture media may cause substrate inhibition and thus lead to a decrease in ethanol production. Many studies on ethanol fermentation technology described that declines in growth and viability of yeasts occurred when sugar concentrations in the media increase from 120 to 180 g/L [9–13], likely because of yeast osmotic stress [11,14]. The effect of osmotic stress on cell metabolism had also been studied by Thomas and coworkers [15], and they deduced that the syntheses of glycolytic enzymes, and the enzymes of the hexose monophosphate pathway, were regulated by sugar concentration. However, Mauricio and Salmon [16], and Salmon and colleagues [17] proved that the principal factor limiting fermentative metabolism was an inhibition of sugar transport. Sugar transport mechanism in yeasts has been characterized by the presence of several transporters that have a specific affinity to glucose. Those transporters presenting high affinity to the substrate that cause catabolic repression; however, they are not detected in fermentation at high sugar concentrations [18,19].

Considering an efficient and economical ethanol production, rapid fermentation is required to produce high ethanol concentrations; therefore, a yeast strain must have a good specific growth rate and specific ethanol production rate at high osmotic stress and ethanol concentrations. Many parameters during batch fermentation will cause a decrease in the specific rate of yeast growth, and inhibition can be caused by either the substrate or the end-product. Other fermentation modes have been attempted to overcome product and substrate inhibition and to improve the ethanol tolerance of yeasts. Among them, the choice of an appropriate process mode and process optimization, such as fed-batch, continuous or semi-continuous [20], and/or manipulation of the composition of cultural media [21], has been one of the most widely explored strategies. The fed-batch culture with the intermittent feeding of glucose and without removal of the fermentation broth, is one of the most common strategies used to produce ethanol in the industry. The advantages of this process mode include the reduction of substrate and end-product inhibition, higher dissolved oxygen rate, higher saccharification rate, decreased fermentation time, and higher productivity of ethanol [22,23]. There have been some studies on the effect of initial glucose concentration as well as fed-batch mode of ethanol fermentation on step-feeding reactor [12,23]; however, we demonstrate a competitive and efficient process as a result of the concomitant increase in cell growth rate and biomass yield, and achieved comparatively higher ethanol performance. The ethanol fermentation processes by batch and fed-batch cultures of *S. cerevisiae* BCRC 21812 using corncob hydrolysate had been compared in our previous report [24]. The results also showed that the fed-batch fermentation had showed a higher ethanol yield than that of the batch fermentation.

The main objective of this work was to develop an economical bioprocess to produce ethanol using an ethanol-tolerant strain of the yeast *S. cerevisiae*, through large-scale laboratory experiments. Fermentative assays were performed to evaluate the effects of initial glucose concentration, residual glucose concentration, yeast biomass on ethanol yield and ethanol conversion rate. The fermentation processes with low (1–10%) and high (15–26%) initial glucose concentrations in the batch and fed-batch cultures of *S. cerevisiae* BCRC 21812 were also compared. High ethanol concentrations and ethanol yields were achieved by the fed-batch mode of culture at the total glucose levels as high as 26%. The growth kinetics of the *S. cerevisiae* during the ethanol fermentation were compared at different glucose concentrations to determine whether it would be feasible for the industry to run fermentation processes at higher sugar concentrations.

2. Materials and Methods

2.1. Microorganisms and Cultivation

The yeast strain, *S. cerevisiae* BCRC 21812, was purchased from the Bioresources Collection & Research Center (Hsinchu City, Taiwan) and used as the inoculum for ethanol fermentation. Yeast cultures were maintained in a DPY medium containing 2% (*w*/*v*) dextrose, 1% (*w*/*v*) peptone and 0.5% (*w*/*v*) yeast extract at 25 °C for 48 h. The initial pH of cultural media was adjusted to 6.5 prior to sterilization at 121 °C for 20 min.

2.2. Media and Fermentations

Yeast for inoculation was grown in Erlenmeyer flasks filled with YMB medium containing (g/L): glucose, 10; peptone, 5; yeast extract, 3; malt extract, 3. After being incubated at 25 °C and being shaken at 140 rpm for 14 h, the yeast cells were inoculated at about 1.0×10^7 cells/mL into the culture medium to initiate the fermentation. The ethanol fermentation was performed in a 5-L stirred fermentor (BTF-5, Biotop Process & Equipment Inc., Taichung, Taiwan) with a 3-L working volume. The batch fermentations were carried out in a medium consisting of 1–26% (*w*/*v*) glucose, 0.5% (*w*/*v*) peptone and 0.25% (*w*/*v*) yeast extract at an initial pH 6.0. The medium for fed-batch fermentation was 18% (*w*/*v*) glucose, 0.5% (*w*/*v*) peptone and 0.25% (*w*/*v*) yeast extract. After 24 h and 48 h, 4% glucose solution was added. Agitation and vortex formation was provided with six-bladed impellers and four side-walled baffle plates. The cultures were agitated at the rates of 600 rpm for 24 h, after which the agitation speed was reduced to 100 rpm. The corresponding aeration rate was initially set at 3.0 vvm for aerobic growth of the yeast and adjusted to 0.5 vvm after 24 h of aeration to create a more anaerobic environment for inducing ethanol production. Dissolved oxygen was measured polarographically by an oxygen electrode. Antifoaming agent was added when required. Samples were drawn at regular intervals from the fermentative broth and analyzed for biomass, pH, glucose and ethanol concentrations. Samples were collected regularly, and levels of glucose and ethanol were analyzed by HPLC.

2.3. Analytical Methods

Samples were withdrawn from the fermentation broth, and yeast biomass was determined by measuring cell optical density, recorded with a Ultrospec 2100 pro-spectrophotometer set at 600 nm (GE Healthcare Co., Barrington, IL, USA). Wet cells were collected by centrifugation at 5000× *g* and were washed with the same volume of distilled water. Dry cell weight was obtained by drying wet cells from a 100 mL culture broth at 100 °C overnight. The reducing sugars liberated by these reactions were measured using the 3,5-dinitrosalicylic acid method of Miller [25], with glucose as the standard.

The concentrations of glucose and ethanol were determined by cation exchange HPLC (Waters Co., Milford, MA, USA) with a 300 mm × 6.5 mm Sugarpak column. Meanwhile, the mobile phase consists of secondary de-ionized water with a flow rate of 0.5 mL/min. All samples, with injection volume set at 20 µL, were filtered through a 0.22 µm filter and then injected into the HPLC column with the column temperature maintained at 90 °C. The HPLC eluate was detected by a refractive index detector

at 50 °C. The ethanol yield (%) was defined as the ratio of the concentration of ethanol produced and glucose consumed.

2.4. Statistical Analysis

The triplicate data were subjected to an analysis of variance for a completely random design using Statistical Analysis System program (SAS Institute Inc., Cary, NC, USA). Comparison of means was analyzed by Duncan's multiple range test and differences were considered significant of $p < 0.05$.

3. Results

3.1. Ethanol Production of the Batch Culture with 4–10% Glucose

To study the effect of glucose concentration on ethanol production, batch fermentation for ethanol production was performed in the media containing various concentrations of glucose as the main carbon source. The time courses of glucose and ethanol concentrations in batch fermentation with initial 4% (w/v) glucose are shown (Figure 1). Glucose was depleted within 20 h, by which time the ethanol concentration and ethanol yield reached 1.6 g/L and 50%, respectively. The average glucose consumption rate was 1.99 g/L/h for 20 h of cultivation. The glucose concentration decreased coincidentally with the increase in cell biomass and ethanol concentration. The yeast cell biomass increased to a maximum of 1.7 g/L and the pH decreased rapidly from 5.5 to 3.5 in the first 18 h of fermentation. The decrease in dissolved oxygen from an initial of 100% to less than 1% after 18 h of incubation created an anaerobic environment, permitting a rapid increase in the production of ethanol. Similar changes were observed for residual glucose, pH, dissolved oxygen content and biomass when the cultures were started with 10% glucose (w/v) (Figure 2). For the initial 12 h of cultivation, the average glucose consumption rate was 1.25 g/L/h when the initial glucose concentration was 10%. During the next 18 h (the period from 12 to 30 h), glucose consumption increased at an average rate of 3.06 g/L/h and the total sugar consumption rate was 2.51 g/L/h. The results showed that the glucose consumption rate in the 10% (w/v) glucose culture was slowed down and exhausted by 30 h of fermentation. The maximal cell biomass (5.1 g/L), ethanol concentration (48.7 g/L) and ethanol yield (50.8%) were achieved by 30 h of fermentation. The maximum values of volumetric cell mass production rate (0.17 g/L/h), specific growth rate (0.18/h) and ethanol production rate (1.62 g/L/h) were found in the cultures with glucose concentration at 10% (w/v) (Table 1).

Table 1. Kinetic parameters for the batch and fed-batch ethanol fermentation.

Fermentation Type	Glucose Concentration (g/L)	Glucose Consumption Rate (g/L/h)	Volumetric Cell Mass Production Rate (g/L/h)	Specific Growth Rate (/h)	Ethanol Production Rate (g/L/h)
Batch fermentation	10	0.71	0.04	0.01	1.02
	40	1.99	0.09	0.05	1.12
	100	3.06	0.17	0.18	1.62
	150	3.13	0.12	0.14	1.61
	180	2.5	0.10	0.09	1.25
	200	2.08	0.07	0.07	1.08
	260	2.09	0.05	0.04	0.96
Fed-batch fermentation	180 + 80	2.17	0.07	0.07	1.08

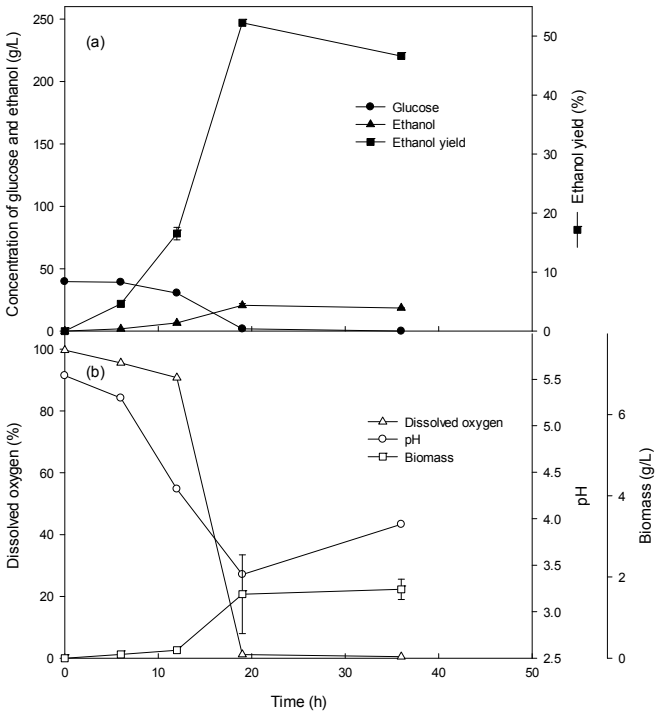

Figure 1. Time course of (**a**) glucose, ethanol concentration, and ethanol yield change, (**b**) dissolved oxygen, pH, and biomass in the batch cultures of *S. cerevisiae* BCRC 21812. The initial glucose concentration was 4% (*w*/*v*).

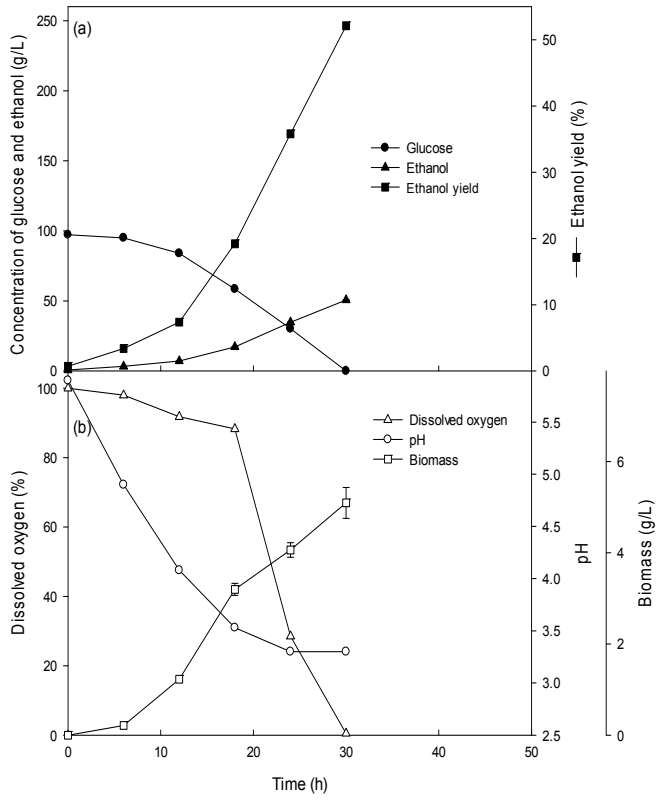

Figure 2. Time course of (**a**) glucose, ethanol concentration, and ethanol yield change, (**b**) dissolved oxygen, pH, and biomass in the batch cultures of *S. cerevisiae* BCRC 21812. The initial glucose concentration was 10% (*w*/*v*).

3.2. Ethanol Production of the Batch Culture with High Concentrations (15–26%) of Glucose

When an initial glucose concentration was elevated to 15% (*w*/*v*), the average glucose consumption rate increased from 2.29 g/L/h to 3.75 g/L/h for the first and next 24 h, respectively. The total sugar consumption rate was 3.13 g/L/h for 48 h of incubation. Glucose consumption rate in the culture initiated with 15% (*w*/*v*) glucose was slowed down and exhausted by 48 h of fermentation (Figure 3). Concentrations of ethanol increased rapidly during the first 48 h of fermentation. The ethanol concentration (77.5 g/L) and ethanol yield (51%) reached the maximum values after 48 h of incubation. It took a much longer fermentation time (48 h) to obtain the maximal cell biomass in the 15% (*w*/*v*) glucose cultures, when compared to the culture that was began with 10% (*w*/*v*) glucose (30 h).

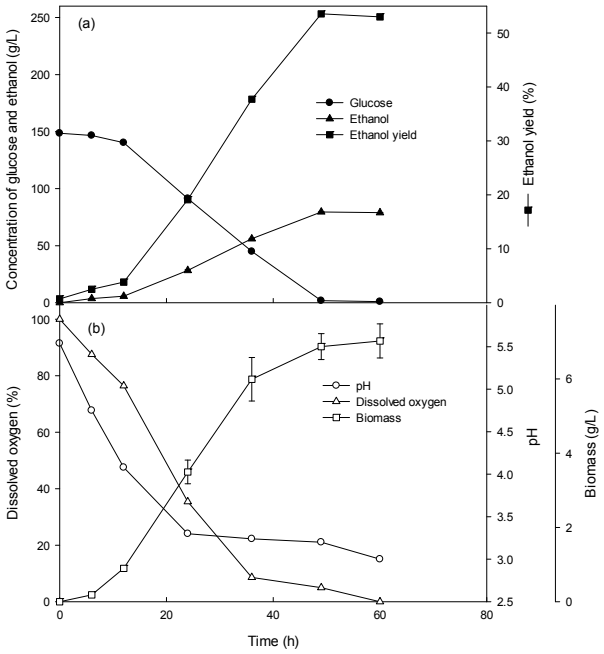

Figure 3. Time course of (**a**) glucose, ethanol concentration, and ethanol yield change, (**b**) dissolved oxygen, pH, and biomass in the batch cultures of *S. cerevisiae* BCRC 21812. The initial glucose concentration was 15% (*w*/*v*).

The time course of the batch fermentation with 18% (*w*/*v*) glucose is shown in Figure 4. The rates of glucose consumption were maintained steadily for 72 h of incubation and the total sugar consumption rate was calculated to be 2.5 g/L/h. It was observed that only minor amounts (0.2 g/L) of residual glucose were detected even after 72 h of fermentation. In addition, the cell biomass of 6.9 g/L, volumetric cell mass production rate of 0.10 g/L/h and specific growth rate (μ) of 0.09/h were found in the cultures with initial glucose concentration at 18%. However, a marked change in the glucose consumption rate was observed in the cultures started with 26% (*w*/*v*) glucose. Glucose depletion was not completely achieved until 120 h (Figure 5). For the initial 72 h of cultivation, the average glucose consumption rate was 2.36 g/L/h. During the next 48 h (the period from 72 to 120 h), glucose consumption decreased at an average rate of 1.67 g/L/h. Therefore, the total sugar consumption rate was 2.09 g/L/h. In addition, the maximal cell biomass, volumetric cell mass production rate and specific growth rate (μ) were significantly decreased to 5.6 g/L, of 0.05 g/L/h and of 0.04/h, respectively. Furthermore, the ethanol yield decreased sharply to 44.7% in the culture with 26% (*w*/*v*) glucose. As shown in Table 1, significant decrease in the rates of the glucose consumption, specific microbial growth, and ethanol production rate were observed at the very high glucose concentration (26%, *w*/*v*) when compared with the cultures with 18% (*w*/*v*) glucose.

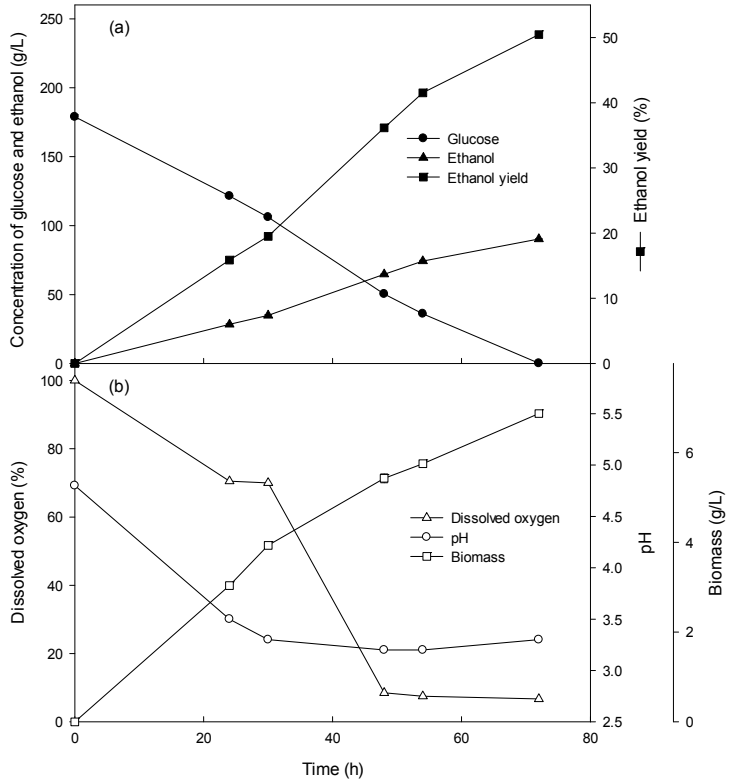

Figure 4. Time course of (**a**) glucose, ethanol concentration, and ethanol yield change, (**b**) dissolved oxygen, pH, and biomass in the batch cultures of *S. cerevisiae* BCRC 21812. The initial glucose concentration was 18% (*w*/*v*).

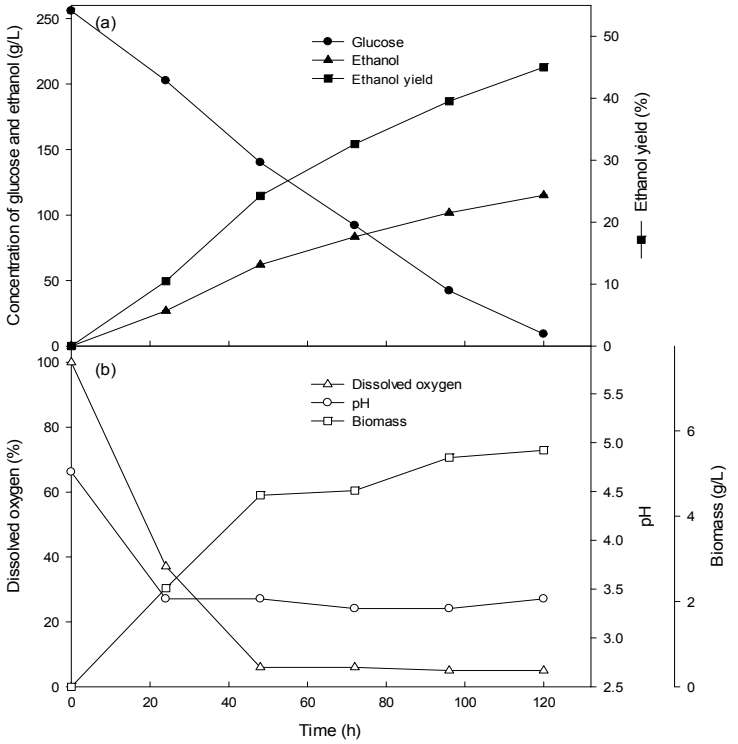

Figure 5. Time course of (**a**) glucose, ethanol concentration, and ethanol yield change, (**b**) dissolved oxygen, pH, and biomass in the batch cultures of *S. cerevisiae* BCRC 21812. The initial glucose concentration was 26% (*w*/*v*).

3.3. Ethanol Production at High Glucose Concentration in the Fed-Batch Culture

The time course of fed-batch fermentation with an initial 18% (*w*/*v*) glucose and addition of 4% (*w*/*v*) glucose after one and two days of incubation was demonstrated in Figure 6. Cell biomass increased gradually throughout the fermentation and reached the maximum of 8.3 g/L. When compared to the batch cultures with initial glucose concentrations of 26% (*w*/*v*), a significant increase in the volumetric cell mass production rate (0.07 g/L/h) and specific growth rate (0.07/h) were found in the fed-batch cultures after 120 h of fermentation. In general, there was no stationary phase of microbial growth observed for the fed-batch culture, at the glucose concentration feeding rates. It was found that glucose consumption rate in the fed-batch culture at the feeding mode was accelerated to 2.17 g/L/h and glucose was exhausted after 120 h of fermentation. Furthermore, ethanol concentrations and theoretical ethanol yield were greatly increased to 130.1 g/L and 100%, respectively (Table 2).

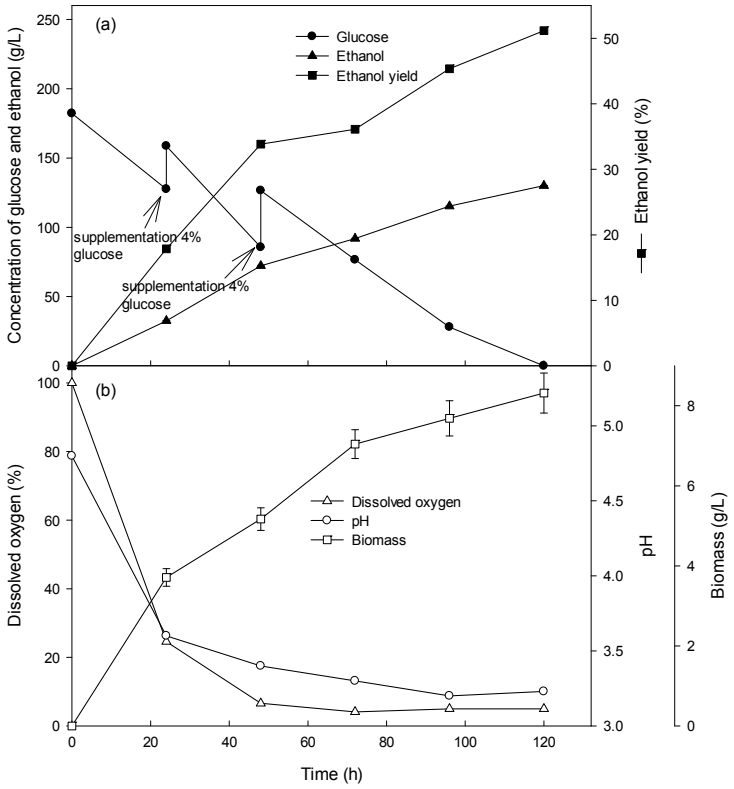

Figure 6. Time course of (**a**) glucose, ethanol concentration, and ethanol yield change, (**b**) dissolved oxygen, pH, and biomass in the fed-batch cultures of *S. cerevisiae* BCRC 21812. The glucose concentration in the fermentation media was initially 18% (*w*/*v*) with an addition of 4% (*w*/*v*) glucose after one and two days of incubation, respectively.

3.4. Comparison of Batch and Fed-Batch Culture for Ethanol Production

Various concentrations of glucose that were used as the sole carbon source for ethanol production in a 5-L fermentor were shown in Table 2. Cell biomass of *S. cerevisiae* BCRC 21812 increased with the increasing concentration of glucose in the range of 1–20% (*w*/*v*). However, biomass concentrations decreased when the glucose concentrations were elevated to 26% (*w*/*v*). In addition, ethanol concentrations increased with the increasing concentration of glucose ranging 1–20% (*w*/*v*). The ethanol concentration was 102 g/L when the initial glucose concentration in the batch culture was 20% (*w*/*v*). However, when the initial glucose concentration reached 26% (*w*/*v*), ethanol concentration (115.1 g/L) did not significantly increase, indicating that the substrate had a considerable inhibitory effect.

Table 2. Comparison of biomass, sugar, ethanol concentration and theoretical ethanol yield of *S. cerevisiae* BCRC 21812 by batch and fed-batch fermentation.

Fermentation Type	Glucose Concentration (g/L)	Residual Glucose Concentration (g/L)	Maximal Cell Biomass (g/L)	Ethanol Concentration (g/L)	Theoretical Ethanol Yield (%)
Batch fermentation	10	ND	0.2 ± 0.0	5.1 ± 0.0	98.4 ± 0.1
	40	ND	1.7 ± 0.1	20.1 ± 0.6	98.2 ± 0.2
	100	ND	5.1 ± 0.2	48.7 ± 0.1	99.6 ± 0.4
	150	ND	7.0 ± 0.4	77.5 ± 0.2	100.0 ± 0.4
	180	0.2 ± 0.0	6.9 ± 0.4	90.3 ± 0.1	98.2 ± 0.3
	200	0.2 ± 0.0	7.0 ± 0.5	101 ± 0.1	97.6 ± 0.4
	260	9.1 ± 0.1	5.6 ± 0.2	115 ± 0.5	87.6 ± 0.6
Fed-batch fermentation	180 + 80	ND	8.3 ± 0.8	130 ± 0.1	100.0 ± 0.2

ND: Not detected.

It was also noted that the residual concentrations of glucose were as high as 9.1 g/L and the ethanol yield decreased to 0.45 g ethanol/g glucose when glucose concentrations in the cultural media were increased to 26% (*w*/*v*). However, a fed-batch mode of culture was performed to diminish the effect of substrate inhibition caused by high glucose concentrations. The initial glucose concentrations in the fed-batch culture were 18% (*w*/*v*) with an addition of 4% (*w*/*v*) glucose after one and two days of incubation, respectively. The results showed ethanol concentrations and conversion rate with the fed-batch cultures increased by 12% and 13%, respectively, when compared to the batch cultures with 26% (*w*/*v*) glucose.

4. Discussion

High ethanol concentrations (130 g/L) and high ethanol yield (0.51 g ethanol/g glucose, 100% of the theoretical value) were achieved by the fed-batch cultures at the feeding concentration of 4% (*w*/*v*) glucose after one and two days of incubation and when final concentrations of glucose in the cultural media were 26% (*w*/*v*). It has been reported that decreases in the growth and viability of yeasts occur as sugar concentrations in the media increase from 12% to 18% (*w*/*v*) [9,11,12]; however, when initial glucose concentrations were higher than 18% (*w*/*v*), they had a considerable inhibitory effect on yeast cell growth, ethanol concentration and ethanol yield. Our studies showed that the inhibitory effect on yeast cell growth as well as ethanol concentration and ethanol yield was diminished by the fed-batch mode of culture.

Theoretically, 1 g of glucose will produce 0.514 g of ethanol and 0.488 g of carbon dioxide. However, in practice, the microorganisms use some of the glucose for growth and the actual yield is less than 100%. It is well known that fermentation efficiency is a key parameter for the industry. Based on the results shown in Table 1, efficient conversion of glucose to ethanol (around 100% of the theoretical value) was achieved for the batch cultures with 10–15% (*w*/*v*) glucose concentrations in the media. The results indicated that a very high efficiency was obtained at high sugar concentrations no greater than 20% (*w*/*v*) by the ethanol fermentation of *S. cerevisiae* BCRC 21812. However, a marked decrease in the ethanol yield to 44.7% (87.6% of the theoretical value) was observed when the initial glucose concentration was increased to 26% (*w*/*v*). The substrate inhibition effect was overcome by the fed-batch culture, which resulted in high ethanol concentration and ethanol yield. In addition, the ethanol yield obtained from our present study indicated efficient conversion of glucose to ethanol by *S. cerevisiae* BCRC 21812. The maximum ethanol yield in this study was significantly higher than those found in many literature reports. For example, Govindaswamy and Vane reported that 5% (*w*/*v*) of initial glucose concentration resulted in 0.49 g ethanol/g glucose of ethanol yield (49%) by *S. cerevisiae* for the duration of 72 h of incubation period [26]. Kumar and colleagues found that an ethanol yield of 40% was obtained in the culture at initial sugar concentrations of 4% (*w*/*v*) [27].

The addition level and feeding times of glucose for the fed batch fermentation were determined from the consumption rate of the batch fermentation. It was observed that glucose was depleted by

yeast after 24 h when the initial concentration of glucose was 4% (*w*/*v*) in the batch culture. From the start of the culture we proposed a strategy to feed glucose into the fermentation media for an interval of 24 h until the end of fermentation; however, extensive feeding of glucose (such as feeding more than 4% per day or total glucose concentration greater than 20%) into the media resulted in decrease in biomass concentration and ethanol yields. As shown in the results by batch cultures, about 4% of glucose for every 24 h intervals was consumed by the yeast cell. The results of our study elucidated that the best protocol for the fed-batch cultures was feeding glucose at concentrations of 4% (*w*/*v*) after one and two days of incubation.

Comparatively, fed-batch cultures produced resulted in a better cell concentration than batch cultures did. Batch cultures in the fermentative media with glucose concentrations greater than 18% demonstrated a smoother type of growth. This growth might be attributed to the osmotic effect caused by the high glucose concentrations, resulting in the slower proliferation of yeast cells [28]. In contrast, the concentration of yeast biomass increased gradually and did not show any stationary phase for the fed-batch culture when initial glucose concentrations of the culture were adjusted to 18% (*w*/*v*) and glucose was added at feeding concentration of 4% (*w*/*v*) of its original concentration after one and two days of incubation. This was probably due to the feeding of 4% (*w*/*v*) glucose, which had provided a better growth environment for the yeast, allowing the yeast cells to divide more rapidly. The higher concentration of substrate was also found to affect the pH, viscosity and the activity of the medium. Exposure to high glucose concentrations for a longer time might also cause catabolic repression. During the fermentation of ethanol, the cell growth rate was observed to be slightly affected by changing the cultural environment. Meanwhile, the increase of sugar concentration caused the decrease of cell viability. These observations were consistent with the results report by Bonin and Skwira [29].

5. Conclusions

In this study, we have demonstrated that maximal ethanol concentration and ethanol yield by a fed-batch culture can be achieved by the feeding mode proposed. Furthermore, we were able to increase the glucose consumption rate efficiently at very high glucose levels and decrease the time needed to obtain the maximal production of ethanol. Ethanol production with fed-batch fermentation offers advantages over production with batch fermentation. The conversion rate of ethanol from glucose was higher in fed-batch fermentation than it was in batch fermentation. Additionally, the inhibitory effects of the substrate on cell biomass and yields of ethanol were less pronounced for fed-batch fermentation than for batch fermentation. Moreover, it was noted that the maximum ethanol concentration and ethanol yield produced was as high as 130 g/L and 51% in 120 h of fermentation. These achievements make the ethanol production process more industrially feasible and efficient. Further studies are focused on how to obtain high levels of ethanol production by applying the fed-batch mode of culture to a scaled-up fermentor.

Author Contributions: H.-D.J., Y.-H.C. and K.-S.C. designed and performed the experiments, derived the models and analyzed the data. C.-Y.C., T.-C.C. and C.-L.H. assisted with the experiments. H.-D.J. wrote the manuscript in consultation with Y.-H.C. and C.-L.H.

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Conflicts of Interest: The authors declare no conflicts of interest.

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