Biotechnology and Bioprocess Engineering **2006**, 11: 72-79

Kinetics of Kojic Acid Fermentation by *Aspergillus flavus* Link S44-1 Using Sucrose as a Carbon Source under Different pH Conditions

M. Rosfarizan* and A. B. Ariff

Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract Kojic acid production by *Aspergillus flavus* strain S44-1 using sucrose as a carbon source was carried out in a 250-mL shake flask and a 2-L stirred tank fermenter. For comparison, production of kojic acid using glucose, fructose and its mixture was also carried out. Kojic acid production in shake flask fermentation was 25.8 g/L using glucose as the sole carbon source, 23.6 g/L with sucrose, and 6.4 g/L from fructose. Reduced kojic acid production (13.5 g/L) was observed when a combination of glucose and fructose was used as a carbon source. The highest production of kojic acid (40.2 g/L) was obtained from 150 g/L sucrose in a 2 L fermenter, while the lowest kojic acid production (10.3 g/L) was seen in fermentation using fructose as the sole carbon source. The experimental data from batch fermentation and resuspended cell system was analysed in order to form the basis for a kinetic model of the process. An unstructured model based on logistic and Luedeking-Piret equations was found suitable to describe the growth, substrate consumption, and efficiency of kojic acid production by *A. flavus* in batch fermentation using sucrose. From this model, it was found that kojic acid production by *A. flavus* was not a growth-associated process. Fermentation without pH control (from an initial culture pH of 3.0) showed higher kojic acid production than single-phase pH-controlled fermentation (pH 2.5, 2.75, and 3.0).

Keywords: kojic acid, sucrose, batch fermentation, modelling, *Aspergillus flavus*

INTRODUCTION

Various types of carbon sources such as glucose, sucrose, xylose, and starch can be used for kojic acid fermentation by fungal strains [6]. It has been reported that *Aspergillus flavus* could be used for direct fermentation of gelatinised sago starch to kojic acid, though production decreased greatly with increasing fermenter volume [12]. However, improvement of production in large-scale fermenters could be achieved through the application of fed-batch fermentation techniques [10]. The use of starch could reduce the cost of raw materials for large-scale production of kojic acid.

An alternative carbon source, one which economically important and is normally used in most fermentation processes, is sucrose. However, information on the use of sucrose as a carbon source for kojic acid fermentation are very limited. Kitada *et al.* [8] reported that kojic acid production by *A. oryzae* using sucrose as a carbon source was very much lower than that from fermentation using glucose. Similarly, kojic acid production by *A. flavus* in shake flask fermentation using sucrose was also comparatively lower than that obtained using glucose [10]. On the other hand, Wei *et al.* [13] reported that high kojic acid production (60 g/L) could be obtained from fermentation by *A. candidus* using 200 g/L sucrose. However, the production of kojic acid was too low to be economically feasible. The yield of product based on sucrose consumed (0.3 g kojic acid/g sucrose) was much lower than that normally obtained in fermentation using glucose, where a value of 0.5 g kojic acid/g glucose has been reported [9]. During fermentation, sucrose is hydrolysed to glucose and fructose by the action of invertase enzymes secreted by kojic acid-producing fungus. Lower kojic acid production obtained in sucrose fermentation by *A. oryzae* and *A. flavus* may be due to the inhibitory effect of sucrose on secretion of enzymes relevant to kojic acid synthesis. Another possibility is the kojic acid-producing fungus is unable to produce a sufficient level of invertase activity for the hydrolysis of sucrose to glucose. Furthermore, reduced kojic acid production has also been reported from fermentation of fructose [8]. Kojic acid production from sucrose may be improved through the development of an appropriate fermentation process. For

***Corresponding author**
Tel: +60-3-8946-7518 Fax: +60-3-8946-7510
e-mail: farizan@biotech.upm.edu.my

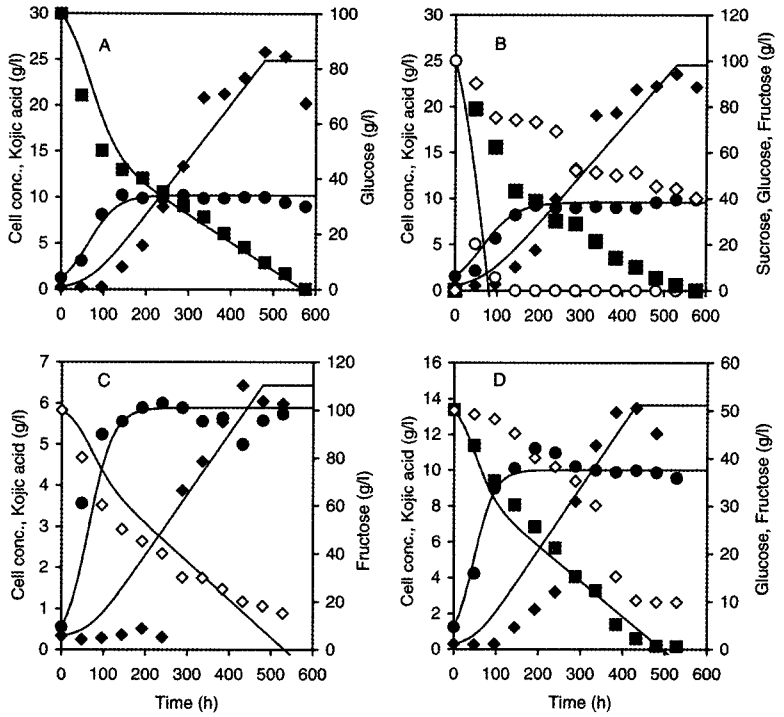

Fig. 1. Kojic acid production by *A. flavus* in shake flask fermentation using various sugars, including the fitness of the calculated values to the experimental data. (A) glucose, (B) sucrose, (C) fructose, and (D) glucose + fructose. (♦) kojic acid; (■) glucose; (•) cell concentration; (◇) fructose; (○) sucrose; solid lines represent calculated data.

Table 1. Kojic acid production and related kinetic parameter values from fermentation of different types of sugar by *A. flavus* in batch culture

Kinetic parameter values	Carbon source			
	Glucose (100 g/L)	Sucrose (100 g/L)	Fructose (100 g/L)	Glucose:Fructose (50:50 g/L)
μ_{max} (h^{-1})	0.030	0.0025	0.035	0.041
x_{max} (g/L)	10.210	9.580	5.890	10
x_0 (g/L)	1.250	1.560	0.560	1.250
p_{max} (g/L)	25.820	23.550	6.430	13.480
p_0 (g/L)	0.246	0.5630	0.336	0.317
α (g carbon/g cell)	5.580	17.580	4.980	1.980
β (g carbon/g cell.h)	0.098	0.098	0.027	0.00074
m (g kojic acid/g cell)	0	0	0	0
n (g kojic acid/g cell.h)	0.0059	0.0055	0.0025	0.0035
$Y_{p/s}$ (g kojic acid/g carbon)	0.260	0.240	0.064	0.013
Overall productivity (g/L.h)	0.054	0.045	0.015	0.031
t[a] (h)	480	528	432	432

[a] Fermentation time was the time needed to reach a maximum kojic acid concentration

this reason, information on the kinetics of fermentation is required.

The present study was carried out to investigate the kinetics of kojic acid fermentation by *A. flavus* using sucrose as a carbon source. The influence of initial sucrose concentration and culture pH on the performance of the fermentation was investigated. For comparison, kojic acid fermentation was also carried out using fructose as a carbon source.

MATERIALS AND METHODS

Microorganism and Medium

The mold used for the production of kojic acid, *A. flavus* Link S44-1, was obtained at the Department of Bioprocess Technology, Universiti Putra Malaysia. Solid medium containing the following ingredients was used for the preparation of slants for spore production: 140 g/L

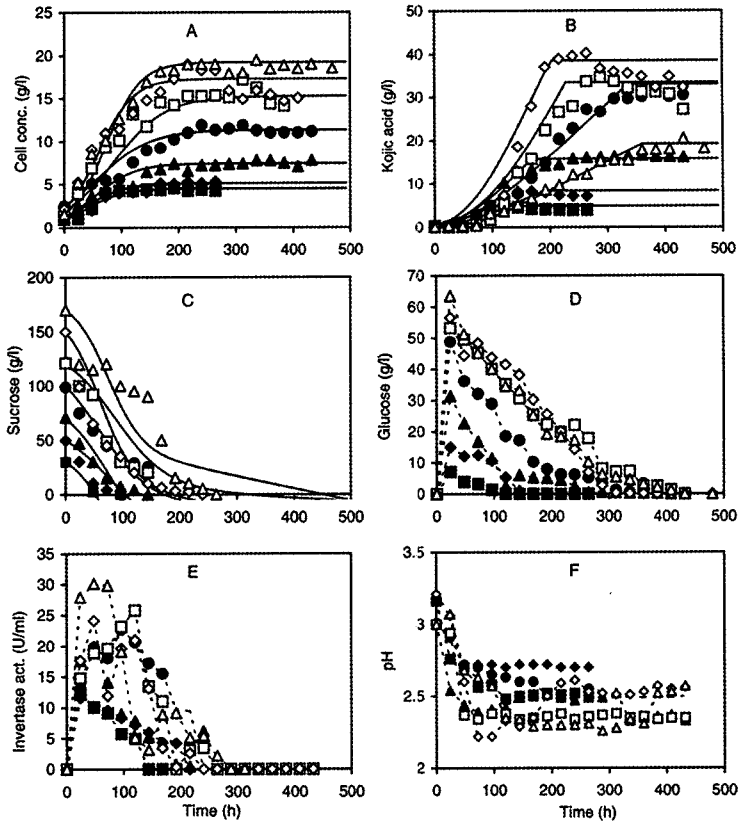

Fig. 2. Batch kojic acid fermentation by *A. flavus* in 2 L stirred tank fermenter using different concentrations of sucrose, with comparisons between calculated and experimental data. (A) cell concentration, (B) kojic acid production, (C) sucrose consumption, (D) glucose concentration, (E) invertase enzyme activity, and (F) pH profile. (■) 30 g/L; (♦) 50 g/L; (▲) 70 g/L; (•) 100 g/L; (□) 120 g/L; (◊) 150 g/L, and (Δ) 170 g/L; solid lines represent calculated data.

sucrose, 1.0 g/L KH_2PO_4, 0.25 g/L $MgSO_4 \cdot 7H_2O$, 2.5 g/L NH_4NO_3, and 15 g/L agar. After inoculation with a loopful of spores from stock culture, the slant was incubated at 30°C for the development of spores. The optimised basal medium for kojic acid production by *A. flavus* as suggested by Ariff *et al.* [2] was used throughout of this study. The medium contained 100 g/L glucose, 5 g/L yeast extract, 1 g/L KH_2PO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, and 10 mL/L methanol.

Fermentation in Shake Flask

To investigate the feasibility of using different types of sugar (sucrose, fructose, and glucose) for kojic acid fermentation, experiments were carried out using 100 g/L of each sugar as a carbon source and 5 g/L yeast extract as the sole nitrogen source. Fermentations were carried out in a 250 mL Erlenmeyer flask containing 100 mL medium with an initial pH of 3. To start the fermentation, 5 mL of spore suspension was inoculated to give an initial spore concentration of approximately 1×10^5 spores/mL medium. Flask cultures were incubated in a rotary shaker agitated at a rate of 250 rpm and a temperature of 30°C. For comparison, fermentation was also carried out using a mixture of sugars (glucose:fructose, 50:50 g/L).

Fermentation in Fermenter

A 2-L stirred tank fermenter (Biostat B, B. Braun, Germany) was used to study the effect of different concentrations of sucrose and yeast extract at a fixed C/N ratio of 93.3 on the performance of kojic acid fermentation by *A. flavus*.

The fermenter was equipped with settings for controlling temperature, pH, and dissolved oxygen. Two six-bladed turbine impellers with a diameter (D) of 52 mm were used for agitation. During fermentation, agitation speed (N) was fixed at 600 rev/min (impeller tip speed = πND = 3.27 m/s) and dissolved oxygen tension (d.o.t) in the culture broth was controlled via a sequential cascade control of airflow rate. The maximum permitted airflow rate was set at 1.5 L/min and the minimum airflow rate set point was 0.1 L/min. The output of the oxygen master controller worked directly on the set point input of the utilized airflow controller. A polarographic dissolved oxygen probe (Ingold) was used to measure d.o.t levels and a steam-sterilizable glass pH electrode (Ingold) was used to monitor culture pH. The sterilized fermenter containing 1.5 L of medium was inoculated with 75 mL of spore suspension to start fermentation with an initial spore suspension of 1×10^5 spore/mL. The initial

Table 2. Yield and kinetic parameter values of kojic acid production in batch fermentation by *A. flavus* using different concentrations of sucrose in a 2-L stirred tank fermenter. Data on fermentation using fructose alone is included for comparison

Kinetic parameter values	Sucrose concentration (g/L)							Fructose (100 g/L)
	30	50	70	100	120	150	170	
μ_{max} (h^{-1})	0.032	0.029	0.029	0.022	0.022	0.033	0.033	0.020
x_{max} (g/L)	4.50	5.12	7.41	11.30	15.26	17.25	19.25	8.97
x_0 (g/L)	0.97	1.23	1.26	2.48	1.98	2.15	1.59	1.58
p_{max} (g/L)	6.82	8.15	16.06	32.15	34.56	40.23	20.50	16.02
Max. invertase act. (U/mL)	12.12	11.61	17.67	22.74	25.80	24.12	30.12	–
α (g sucrose/g cell)	1.58	6.58	10.58	12.25	8.58	9.25	7.26	11.69
β (g sucrose/g cell.h) × 10^{-2}	29.80	39.20	8.00	0.018	0.198	0.578	0.583	0.198
m (g kojic acid/g cell)	0	0	0	0	0	0	0	0
n (g kojic acid/g cell.h) × 10^{-2}	1.89	5.89	1.89	1.09	1.59	1.59	0.35	0.59
$Y_{p/s}$ (g kojic acid/g sucrose)	0.23	0.163	0.23	0.32	0.29	0.27	0.12	0.16
Overall productivity (g/L.h)	0.057	0.057	0.061	0.084	0.12	0.152	0.047	0.034
t[a] (h)	120	144	264	384	288	264	432	480

[a] Fermentation time was the time needed to reach a maximum kojic acid concentration.

pH of the culture was adjusted to 3.0 and the culture pH was not controlled during fermentation. The d.o.t level during the fermentation was recorded continuously by using a recorder connected to the main controller. The optimum aeration control strategy for maximum batch production of kojic acid was employed [2]. In this control strategy, the d.o.t was controlled at 80% saturation during the growth phase and at 30% saturation during the production phase (when growth reached a stationary phase). The temperature within the fermenter was maintained at 30°C.

The effect of fermentations with controlled pH (pH 2.5, 2.75, and 3.0) on kojic acid production was also carried out using 150 g/L sucrose as a carbon source and 7.5 g/L yeast extract as a nitrogen source.

Analytical Methods

During fermentation and reaction, samples were withdrawn at various time intervals for analysis. Samples were filtered using preweighed microfiber filters. The supernatants were used for kojic acid and glucose determination, while the solid residues were dried in an oven at 95°C for dry cell weight measurement.

Kojic acid concentration was determined by using high performance liquid chromatography (HPLC) with ultraviolet detector at 265 nm as described previously [1]. Glucose, sucrose and fructose concentrations in the culture broth were determined using HPLC (Shimadzu LC10AS) with refractive index detector (Shimadzu RID-6A). Separation of sugars was obtained using an NH_2 Column (Merck) eluted with 80% acetonitrile in deionised water. The column was maintained at room temperature and at a flow rate of 1.0 mL/min.

Mathematical Method

The following simplified batch fermentation kinetic models for cell growth, substrate consumption, and product formation were based on logistic and Luedeking-Piret equations [1,14]. These models were used to evaluate the kinetics of kojic acid fermentation by *A. flavus* Link using sucrose as a carbon source.

Cell growth:

$$dx/dt = \mu_{max}(1-x/x_{max}) \quad (1)$$

Substrate consumption:

$$ds/dt = \alpha(dx/dt) + \beta x \quad (2)$$

Product formation:

$$dp/dt = m(dx/dt) + nx \quad (3)$$

The kinetic models (Eqs. 1 to 3) were fitted to the experimental data by non-linear regression with a Marquadt algorithm using MATLAB computer software. The model parameter values were first evaluated by solving Eqs. 1 to 3 and then the computer program was used as a search method to minimize the sum of squares of the differences between the predicted and measured values. The predicted values were then used to simulate the profiles of cell, substrate, and product concentrations during fermentation. In order to determine the significance of deviations between experimental and calculated data, statistical analysis (unpaired t-test) was also carried out.

RESULTS AND DISCUSSION

Fermentation in Shake Flask

Fig. 1 shows a typical time course of kojic acid fermentation in a shake flask using different types of sugar: glucose, sucrose, fructose, and a mixture of glucose and fructose (A-D). This figure also includes simulation curves calculated according to the proposed kinetic models. The models fit fairly well, with more than 93% confidence, to the experimental data. Comparisons of the per-

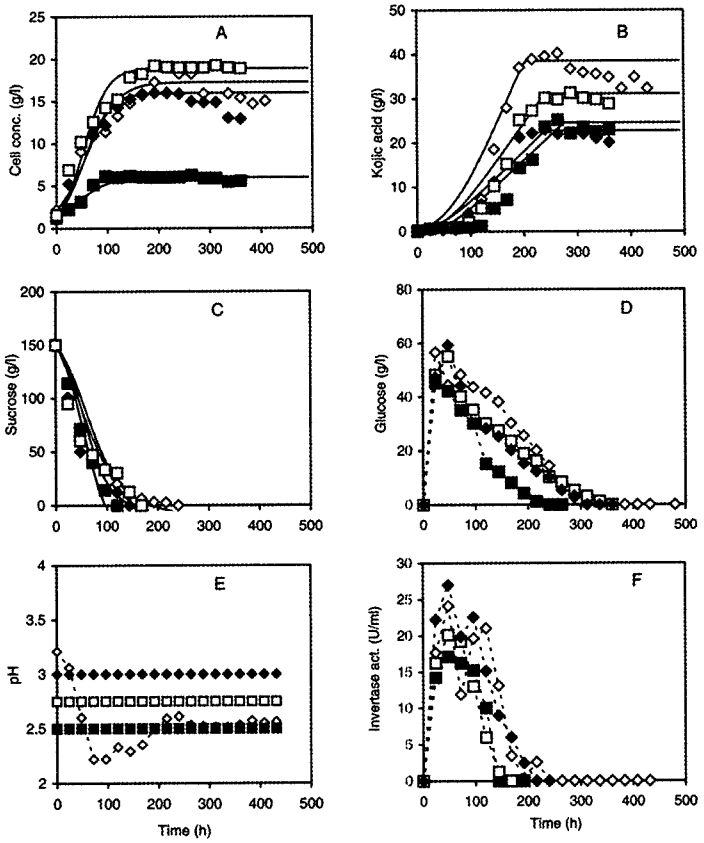

Fig. 3. The pH-controlled kojic acid batch fermentation by *A. flavus*, including comparison between calculated and experimental data. (A) cell concentration, (B) kojic acid production, (C) sucrose consumption, (D) glucose concentration, (E) pH profile, and (F) invertase enzyme activity. (■) pH 2.5; (□) pH 2.75; (♦) pH 3.0, and (◊) initial pH 3.0; solid lines represent calculated data.

formance and the kinetic parameter values of using different types of sugar for kojic acid fermentation in a shake flask culture are shown in Table 1. The kinetic parameter values obtained can be used to verify experimental data on kojic acid production by *A. flavus* using various sugars. In this study, it was found that kojic acid production from sucrose and fructose was not a growth-related process, as indicated by the zero value of growth-associated rate constants for kojic acid formation (m) in all fermentations. The non-growth-associated rate constant for kojic acid formation (n) from sucrose was comparable to that from glucose. These values were about two times higher than that observed for fermentation using fructose. As in fermentation using glucose, excellent growth was also obtained from fermentations using sucrose alone, and from a mixture of glucose and fructose. Growth was much lower from fermentation using fructose, suggesting that fructose was a poor carbon source for the growth of *A. flavus*. However, the maximum specific growth rate (μ_{max}), with an average value of 0.035 h^{-1}, was not significantly different between the different sugars used.

The highest kojic acid production was obtained from fermentation using glucose (25.8 g/L), which gave an overall productivity of 0.054 g/L.h. The *A. flavus* fungus was capable to utilise sucrose for growth and kojic acid production, indicating that the fungus secreted sufficient amounts of invertase for the hydrolysis of sucrose to glucose and fructose under the prevailing fermentation conditions. However, the fermentation time (528 h) was about 48 h longer than for fermentation using glucose. In addition, kojic acid production (23.6 g/L) and productivity (0.045 g/L.h) for fermentation using sucrose were slightly lower than that from fermentation using glucose. In kojic acid fermentation by *A. oryzae* using 100 g/L sucrose, the maximum kojic acid concentration obtained was 1.4 g/L [8]. On the other hand, high kojic acid production (60 g/L) was obtained from fermentation by *A. candidus* using 200 g/L sucrose [13]. Very low kojic acid production (6.4 g/L) was obtained from fermentation using fructose, though the fermentation time (432 h) was about 48 h shorter than fermentation using glucose. From this study, it was found that fructose was a poor sugar for growth of *A. flavus* and kojic acid production. Kitada *et al.* [8] reported that kojic acid was not produced by fermentation by *A. oryzae* using fructose, though fungal growth was excellent. Fructose is well known as a poor carbon source for kojic acid production because its furanose form is not suitable for direct conversion to kojic acid [7]. In fermentation using a mixture

Table 3. Yield and kinetic parameter values of batch kojic acid fermentation by *A. flavus* in a 2-L stirred tank fermenter at various controlled pH and without pH controls

Kinetic parameter values	Single phase pH-controlled			Without pH control (Initial pH 3.0)
	pH 2.5	PH 2.75	pH 3.0	
μ_{max} (h^{-1})	0.033	0.041	0.044	0.033
x_{max} (g/L)	5.99	15.98	18.89	17.25
x_0 (g/L)	1.25	1.56	1.58	2.15
p_{max} (g/L)	22.98	31.23	25.14	40.23
Max. invertase act. (U/mL)	17.12	20.12	26.98	24.12
α (g sucrose/g cell)	22.25	12.25	8.25	9.25
β (g sucrose/g cell.h)	0.078	0.00538	0.00528	0.00578
m (g kojic acid/g cell)	0	0	0	0
n (g kojic acid/g cell.h)	0.0195	0.0105	0.0059	0.0159
$Y_{p/s}$ (g kojic acid/g sucrose)	0.23	0.31	0.25	0.40
Overall productivity (g/L.h)	0.096	0.108	0.095	0.152
t^a (h)	240	288	264	264

[a] Fermentation time was the time needed to reach a maximum kojic acid concentration.

of glucose (50 g/L) and fructose (50 g/L), kojic acid production (13.5 g/L) was only about half that obtained by fermentation of either glucose or sucrose alone. This indicates that fructose did not inhibit kojic acid production. It seemed that in this case, the enzymes relevant to kojic acid synthesis were not capable to convert fructose to kojic acid. Most of the kojic acid produced from this fermentation may be from glucose.

Fermentation in a 2-L Stirred Tank Fermenter

Effect of Sucrose Concentration

Fig. 2 (A-F) shows the profile of growth, sucrose hydrolysis, glucose accumulation, invertase activity, kojic acid production, and pH during the fermentation of *A. flavus* using different sucrose concentrations. In all cases, growth was very rapid during the early stages of fermentation and reached a stationary phase after about 120 h. During the rapid growth phase, sucrose was hydrolysed to glucose and fructose by the invertase enzyme secreted by the fungus. The production of invertase was significantly affected by sucrose concentration. In general, invertase production was directly proportional to the concentration of sucrose. The activity produced was sufficient to hydrolyse all the sucrose supplied to the culture, suggesting that the rate and degree of sucrose hydrolysis was not a major problem in kojic acid fermentation by *A. flavus*. A rapid decrease in invertase activity was observed during fermentation, indicating that prevailing fermentation conditions were not appropriate for the stability of invertase. In all cases, no invertase activity was detected toward the end of the production phase.

Maximum cell concentration (x_{max}) attained during the fermentation increased with increasing sucrose concentration. The highest maximum cell concentration (19.3 g/L) was obtained in fermentation using 170 g/L sucrose. At the same time, kojic acid production increased almost linearly with increasing sucrose concentration up to 150 g/L. Within the range of sucrose concentration studied, the highest kojic acid production (40.2 g/L) was obtained from fermentation of 150 g/L sucrose, with an overall productivity of 0.152 g/L.h. These values were slightly higher than those obtained from fermentation using 100 g/L glucose. In terms of yield efficiency, fermentation using sucrose showed lower values (0.27 g kojic acid/g sucrose) than fermentation using glucose (0.35 g kojic acid/g glucose). Rate and degree of sucrose hydrolysis in fermentation were not a major problem even using high sucrose concentration. In view of this fact, reduced kojic acid production in fermentation using high sucrose concentration (170 g/L) could be due to inhibition by sucrose of secretion of enzymes relevant to kojic acid during the growth phase. Fermentation of 100 g/L sucrose in a 2-L fermenter was about 144 h shorter than shake flask fermentation.

Kojic acid production (16.0 g/L) and maximum cell concentration (9.0 g/L) were low in fermentation using fructose alone. However, the values were about two times higher in the fermenter than those obtained in shake flask culture. It is interesting to note that the fermentation time for fermentaion in the 2-L fermenter was slightly longer than the time taken for fermentation in shake flask. In termed of overall productivity, the value for fermentation in 2-L fermenter (0.034 g/L.h) was about two times higher than that for shake flask fermentation. This result indicates that the optimal aeration control strategy and hydrodynamic conditions within the fermenter may be important for the improvement of kojic acid production using fructose as a carbon source.

Fig. 2 (A-C) also shows the comparison of calculated and experimental data for fermentation by *A. flavus* in a 2 L stirred tank fermenter using different concentrations of sucrose. The performance and kinetic parameter values of all fermentations are presented in Table 2. In all cases, the models fit well with experimental data on growth, sucrose hydrolysis, and kojic acid production. The maximum specific growth rate (μ_{max}) was not significantly different for the different concentrations of sucrose used.

However, the growth value (μ_{max}) of *A. flavus* in a 2-L fermenter using sucrose was about three times lower than the growth value from using glucose. The value of the growth-associated rate constant for sucrose consumption (α) increased with increasing sucrose concentration up to 100 g/L and levelled off between 120~170 g/L sucrose. On the other hand, the value of the non-growth-associated rate constant for sucrose consumption (β) was very high at low sucrose concentrations (30~50 g/L) but was consistently low at sucrose concentrations ranging from 100 to 170 g/L. The value of non-growth-associated rate constant for kojic acid production (n) was the highest (0.0589 g kojic acid/g cell.h) for fermentation of 50 g/L sucrose. The value was not significantly different for fermentation of sucrose from 70 to 150 g/L, with an average value of about 0.0154 g kojic acid/g cell.h and reduced greatly for fermentation of 170 g/L. The value for fermentation of 170 g/L sucrose was only about 1/3 the value for fermentation of 150 g/L sucrose. This observation indicates that the fungal cells cultivated at high sucrose concentration have lower activity of the cell-bound enzyme system responsible for kojic acid synthesis, which in turn, reduced the efficiency in converting sugar to kojic acid. Kojic acid production was dependent on the cell bound enzyme system responsible for kojic acid synthesis [3,4,5]. The secretion of these enzymes may be subject to carbon catabolite repression. Such repression normally controls the expression of many structural genes involved in carbon source utilisation that affect enzyme secretion. High sucrose concentration may repress the secretion of enzymes relevant to kojic acid synthesis. The hydrolysis of sucrose could be limited by insufficient activity of invertase in the culture.

Effect of pH-controlled fermentation

The effect of pH on growth of *A. flavus*, kojic acid production, sucrose hydrolysis, glucose production, and invertase activity are shown in Fig. 3 (A-F), which also shows the fitness of the calculated data to the experimental data. The comparison of the performance and the kinetic parameter values for kojic acid production with and without pH control is summarised in Table 3. The fermentation of sucrose to kojic acid by *A. flavus* was greatly influenced by pH. The maximum specific growth rate (μ_{max}) was increased with increasing pH from 2.5 to 3.0. However, the value of maximum specific growth rate from fermentation without pH controls (from an initial culture pH of 3.0) was similar to that for fermentation where the pH was controlled at 2.5. The maximum cell concentration obtained in the pH-controlled fermentation at 2.5 (6.0 g/L) was very much lower than pH-controlled fermentation at 2.75 (16.0 g/L). This indicated that growth of *A. flavus* was greatly affected by even very slight changes in pH. In other words, growth of *A. flavus* was greatly inhibited at pH below 2.75.

The secretion of invertase by *A. flavus* was not greatly influenced by pH. There was no significant difference in invertase activity during fermentation where pH was controlled at 2.75, 3.0, and at uncontrolled pH control. Reduced invertase production was observed in fermentation controlled at a pH of 2.5, suggesting that invertase production was inhibited at very low pH during the growth phase. The amount of glucose accumulated in pH-controlled fermentation was lower than that obtained from uncontrolled fermentation, which was related to the low activity of invertase in the culture during the fermentation.

Controlled changes in pH between 2.5 and 3.0 did not highly influence fermentation of sucrose to kojic acid by *A. flavus*. Fermentation at a controlled pH of 2.75 gave the highest kojic acid production (31.2 g/L). However, the efficiency of cells to synthesise kojic acid, as indicated by the value of n, was the highest at pH 2.5. The results from this study also showed that the fermentation in cultures without pH controls (from an initial culture pH of 3.0) produced significantly higher amounts of kojic acid (40.2 g/L) than a single-phase pH-controlled fermentation. It seemed that *A. flavus* was able to adjust the surrounding pH naturally for enhancement of kojic acid production, especially during the growth phase. In other words, the addition of acid and alkali to control the culture pH was not appropriate for the activity of the fungus toward enhancement of kojic acid production. In contrast, kojic acid production from glucose by *A. flavus* was improved by about 20% in fermentation with a pH control strategy though the time to reach maximum concentration was the same as that without pH controls [10].

The value of the non-growth-associated rate constant for kojic acid production (n) for pH-controlled fermentation at 2.75 and 3.0 was lower than the value for fermentation without pH control. Very high n values were obtained for fermentation where pH was controlled at 2.5, indicating that the fungal cells have a high ability to synthesize kojic acid. The value of growth-associated rate constant (α) at a controlled fermentation pH of 2.5 was more than twice that at pH 2.75 and almost 4 times higher than the value of this constant at pH 3.0. The value of the non-growth associated rate constant (β) for glucose consumption at pH 2.5 was 45% higher than for pH-controlled fermentation at 2.75 and 50% higher than that at a pH of 3.0. There was little difference in the value of α between fermentation at a controlled pH of 2.75, at a controlled pH of 3.0, and without pH controls. β was more or less the same in this regard as well. From this study, it was found that the influence of pH on kojic acid fermentation using sucrose was much grater during the growth phase than in the production phase. Several enzymes were found to be relevant to kojic acid synthesis by *A. flavus*: glucose dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phophogluconate dehydrogenase, and hexokinase [5]. In fermentation using glucose, an initial pH of 3.0 without subsequent regulation of pH during the growth phase was favourable for the production of cell materials containing high activities of enzymes relevant to kojic acid synthesis [11].

CONCLUSION

A. flavus was capable to utilise sucrose for growth and

kojic acid production. The optimal concentration of sucrose for kojic acid fermentation was 150 g/L. The proposed models based on logistic and Luedeking-Piret equations were found suitable to describe kojic acid fermentation at various concentrations of sucrose and different pHs. The models can be used to generate parameter kinetic values that may be used to verify experimental data and to make simulations. Fermentation without pH control (initiated at culture pH 3.0) was found to be more appropriate for kojic acid production than single-phase pH-controlled fermentation at pH 2.5, 2.75, and 3.0. Using optimal concentrations of sucrose, batch fermentation without pH controls produced slightly higher kojic acid (40.2 g/L) levels than fermentation using glucose. In terms of overall productivity, fermentation using sucrose (0.152 g/L.h) was comparable to glucose (0.133 g/L). However, fermentation using glucose gave a slightly higher yield (0.35 g kojic acid/g glucose) than fermentation using sucrose (0.27 g kojic acid/ g sucrose).

NOMENCLATURE

α	Growth-associated rate constant for glucose consumption (g glucose/g cell)
β	Non-growth-associated rate constant for glucose consumption (g glucose/g cell.h)
m	Growth-associated rate constant for kojic acid production (g kojic acid/g cell)
n	Non-growth associated rate constant for kojic acid production (g kojic acid/g cell.h)
x	Cell concentration (g/L)
x_{max}	Maximum cell concentration (g/L)
t	Time (h)
s	Substrate concentration (g/L)
p	Kojic acid concentration (g/L)

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[Received November 17, 2005; accepted February 3, 2006]