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Effect of Vacuum Pressure on Ethanol Fermentation

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Abstract: This study presents batch ethanol fermentation processes at various vacuum pressures. Glucose was used as substrate to anaerobically produce ethanol using baker's yeast at 47-760 mmHg and 33°C. The results indicated that the baker's yeast can survive and convert glucose to ethanol without oxygen/air supply while the carbon dioxide generated, can be trapped by liquid nitrogen, thus allowing vacuum pressure to be maintained without running vacuum pump continuously. The advantage of fermentation under vacuum pressure comes from the high ethanol concentration collected in the cold-trap while maintaining the productivity of fermentation processes in a range of 1.7 to 2.2 g/L/h. When increasing fermentation time, the productivity at low pressure was increased and higher than those at atmospheric pressure. A significant amount of ethanol outlet product was collected in the cold-trap. This ethanol concentration increased while increasing fermentation time and reached approximately 50% by weight at the end of fermentation period corresponding to system pressure of 47 mmHg. A kinetic model for ethanol fermentation under vacuum was developed to investigate the effect of vacuum pressure on the growth rate and production rate of the baker's yeast. This kinetic model and its set of rate equation parameters were developed and validated by experimental data of batch fermentation processes at atmospheric pressure.

Key words: Kinetic model, vacuum ethanol fermentation, baker's yeast, substrate, product inhibition

INTRODUCTION

High-concentration ethanol fermentation processes are of significant interest because significant energy savings can be achieved for downstream distillation and waste stillage treatment (Bai *et al*., 2008). Fermentation processes are inhibited by the high concentrations of substrate and products. In the case of ethanol fermentation from glucose, the limitation of conventional process comes from high initial glucose concentration and high ethanol concentration inhibition. When initial glucose concentration in the medium is over 300 g L^{-1} and/or ethanol concentration in the fermentation broth reaches 15% by volume, both a specific growth rate and specific production rates of yeast decline, the cell mass in the fermentation broth decreases and glucose cannot be converted completely to ethanol (Cysewski and Wilke, 1977). This limitation of substrate or product inhibition can be solved by the integration of fermentor with separator. This integration method satisfies two objectives: (1) preventing product inhibition by reducing ethanol concentration in the fermentation broth and (2) preventing the decline of yeast cells at high substrate concentration by recycling cells back to fermentor.

For preventing product inhibition, ethanol should be removed from the fermentation broth as it is formed. Many proposed configurations using this integration approach are related to ethanol removal by different means such as vacuum fermentation, gas tripping, pervaporation combining fermentation and liquid extraction. Among them, the vacuum fermentation has been widely investigated for the ethanol fermentation with *S. cerevisiae* and *Z. mobilis* (Cysewski and Wilke, 1977; Ghose *et al*., 1984; Le *et al*., 1981; Ishida and Shimizu, 1996). The researches have shown that the fermentation under vacuum pressure is an effective alternative method for continuous ethanol removal. Basically, the theory of fermentation process running under vacuum pressure is based on both physical properties of ethanol-water mixture and biochemical properties of fermentation reaction by yeast. The fermentation process by yeast is carried out in a temperature of 30 to 35°C and the mixture of ethanol-water boils at 78.3 to 100°C. When the fermentation process is operated under vacuum pressure,

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the boiling point temperature of this mixture decreases. However, this integration approach does not solve some practical limitations such as the high of energy consumption for maintaining the vacuum condition and their high working volumes of fermentors for operating under vacuum.

To overcome these limitations, this study proposed an alternative operating condition for fermentation under vacuum pressure. This study showed that the yeast cells can survive and grow with very little amount of oxygen when the fermentation process is operated under vacuum pressure. In addition, the amount of sub-saturated carbon dioxide can be trapped almost by liquid nitrogen, so that vacuum condition can be maintained in the fermentor without running vacuum pump continuously.

MATERIALS AND METHODS

Microorganism, media and methodology: Commercially available baker's yeast, *Saccharomyces cerevisiae*, supplied by Aldrich Co. (Germany) was used as inoculum. Pre-culture of yeast cells was carried out in 250 mL Erlenmeyer flask containing 100 mL medium composed of 2 g glucose; 1 g yeast extract; 2 g peptone. Stirrer speed and temperature were controlled at a suitable level and 30°C within 12 h. Then, the 30 mL of the pre-culture was transferred to 670 mL of the culture medium. This culture medium was mixed by stirrer and submerged into water bath with temperature controller. The culture medium contained 100 g L^{-1} glucose, 8.5 g L^{-1} yeast extract, 1.25 g L^{-1} NH_4Cl, 0.06 g L^{-1} $CaCl_2$, 3.25 g L^{-1} $(NH_4)_2SO_4$ and 0.11 g L^{-1} $MgSO_4$. When the glucose concentration of the culture medium was changed, all other components were changed by the same ratio. Both media were sterilized at 121°C for 15 min. The pH of the fermentation broth was maintained by adding drops of 3N NaOH solution. The fermentation liquid was analyzed to determine the glucose concentration, ethanol concentration and cell dried mass. The vapor sample in vapor phase and the vapor condensate sample in the cold-trap are analyzed to determine the carbon dioxide in vapor phase and ethanol concentration in accumulated condensate.

Analytical methods: Ethanol was measured by gas chromatography (Shimadzu, GC-8A) using a Thermal Conductivity Detector (TCD) and a porapak-Q packing column. The carrier gas was helium with flow rate of 25 mL min^{-1}. Residual glucose was determined by the Dinitrosalicylic acid method (Sumner and Somers, 1944). A UV-VIS spectrophotometer (model V-6300, Jusco, Tokyo, Japan) at 495 nm was used to measure the absorbance. The mass of dry yeast cells were measured and correlated with optical density determined using the same UV-VIS spectrophotometer at 501 nm.

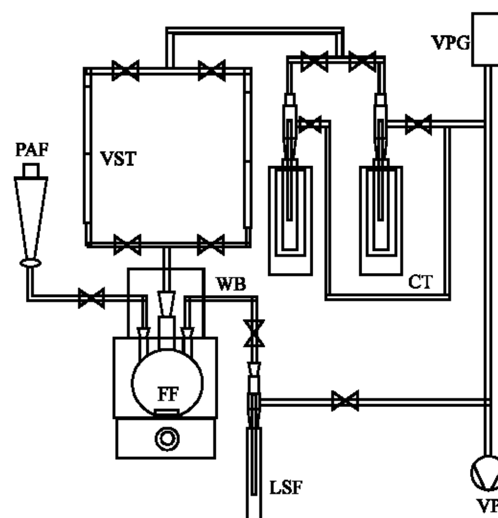

Fig. 1: Schematic of the batch fermentation process under vacuum pressure, WB: Water batch with temperature indicator, FF: Fermenter flask, VST: Vapor sampling tube, LSF: Liquid sampling tube, CT: Cold trapper, PAF: pH adjustment flask, VPG: Vacuum pressure guage, VP: Vacuum pump

Experimental process: The schematic diagram of the batch fermentation set-up under vacuum pressure is shown in Fig. 1. Seven hundred milliliter medium and culture were added to the Fermenter Flask (FF). The fermentation temperature was maintained at 33°C by a Water Bath (WB). The system pressure was reduced from 760 mmHg to a desired pressure by a Vacuum Pump (VP). The reduction speed was 25 mmHg min^{-1}. The stable condition of system is guaranteed by needle valves. The vapor sample in Vapor Sampling Tube (VST), liquid sample in Cold-Trap (CT) and broth sample in Liquid Sampling Flask (LSF) were taken every 5-6 h during fermentation period. These samples were used to analyze carbon dioxide in vapor phase, ethanol concentrations in cold-trap, glucose, ethanol and cell dried mass concentration in fermentation broth. The set-up was sterilized in place by filling a recycling amount of the 70% ethanol-water solution and boiling the solution under atmospheric pressure for 1 h. The remaining amount of the sterilizing solution was then removed by dying with air supplied by an air pump for 30 min. The system was protected by the air filter (⌀ = 0.1 μm). The fermentation medium and sodium hydroxide solution were sterilized in autoclave at 121°C for 15 min. Batch fermentation

experiments under various pressures of 760, 100, 65 and 47 mmHg and 33°C were investigated. The initial glucose was about 250 g L^{-1}.

Kinetic model and parameter determination of fermentation process under vacuum pressure: A mass balance was carried out on the ethanol batch fermentation system under vacuum condition to determine the cell growth, substrate utilization and ethanol production rates, as shown in the following:

$$\frac{dVX}{dt} = V\frac{dX}{dt} + X\frac{dV}{dt} = \mu XV \quad (1)$$

$$V = V_0 - F_{ave}t \quad (2)$$

where, F_{ave} (L h^{-1}) is an average evaporating flow rate, V (L) is fermentor volume, V_0 (L) is fermentor volume at t = 0, X (g L^{-1}) is cell dry mass, μ h^{-1} is specific growth rate.

Substituting Eq. 2 in Eq. 1 yields,

$$(V_0 - F_{ave}t)\frac{dX}{dt} - XF_{ave} = \mu X(V_0 - F_{ave}t) \quad (3)$$

The rate of the product formation can be calculated as follows:

$$\frac{dVP}{dt} = V\frac{dP}{dt} + P\frac{dV}{dt} = \nu XV - F_{ave}P_{ave} \quad (4)$$

where, P (g L^{-1}) is ethanol concentration in liquid phase; P_{ave} (g L^{-1}) is average ethanol concentration in vapor phase, ν h^{-1} is specific production rate.

The vapor-liquid equilibrium of the ethanol-water mixture is calculated by Eq. 5, the value of π_i^{sat} is calculated by Antoine's equation and the value of γ_i is calculated using Wilson method (Smith *et al*., 2001).

$$K_i = \frac{P_{ave}}{P} = \gamma_i \frac{\pi_i^{sat}}{\pi} \quad (5)$$

Substituting Eq. 2 in Eq. 4 yields,

$$(V_0 - F_{ave}t)\frac{dP}{dt} - PF_{ave} = \nu X(V_0 - F_{ave}t) - F_{ave}P_{ave} \quad (6)$$

The rate of the substrate utilization can be calculated as follows:

$$-\frac{dVS}{dt} = -V\frac{dS}{dt} - S\frac{dV}{dt} = \frac{1}{Y_{P/S}}\frac{dVP}{dt} = \frac{1}{Y_{P/S}}(\nu XV - F_{ave}P_{ave}) \quad (7)$$

where, S (g L^{-1}) is glucose concentration, $Y_{p/s}$ is ethanol yield factor.

Substituting Eq. 2 in Eq. 7 yields the following Eq. 8:

$$-(V_0 - F_{ave}t)\frac{dS}{dt} - SF_{ave} = \frac{1}{Y_{P/S}}(\nu X(V_0 - F_{ave}t) - F_{ave}P_{ave}) \quad (8)$$

The kinetic equation for specific growth rate (μ) and specific production rate (ν) of yeast cells obtained were as follows:

$$\mu = \mu_m \frac{S}{S + K_S + \frac{S^2}{K_i}}\left(1 - \frac{P}{P_m}\right) \quad (9)$$

$$\nu = \nu_{max} \frac{S}{S + K_S' + S^2/K_i'}\left(1 - \frac{P}{P_m'}\right) \quad (10)$$

In Eq. 9 and 10, μ_{max} (h^{-1}) is the maximum specific growth rate, K_S and K'_S (g L^{-1}) are saturated constants, K_i and K_i' (g L^{-1}) are inhibition constants, P_m (g L^{-1}) is the threshold ethanol concentration above which cell concentration decreases, ν_{max} h^{-1} is the maximum specific production rate and P_m' (g L^{-1}) is the threshold ethanol concentration above which dp/dt is closed to zero. $Y_{p/s}$ is defined as ethanol yield factor of ethanol's production and glucose's utilization.

$$Y_{P/S} = \frac{\text{Mass of ethanol produced}}{\text{Mass of glucose consumed}} = -\frac{dP/dt}{dS/dt} \quad (11)$$

In order to investigate the effect of vacuum pressure, the following kinetic parameters: μ_m, K_s, K_i, P_m, ν_m, K_s', K_i', P_m' and $Y_{p/s}$ were determined depending on vacuum pressure. Since, the model was a non-linear model with multi-parameters, the optimization for the set of parameter significantly depended on the initial guesses for the parameter values.

Initial parameter values were tentatively estimated from the batch experimental data at atmospheric pressure and various initial glucose concentrations (Nguyen *et al*., 2009). The initial kinetic parameters were then re-calculated by running the program iterations. The best-fit values of the parameters were estimated using Sum of the Square of Error (SSE) technique between the estimated and experimental data. The differential equations from the developed model were solved using the ODE45 (®MATLAB 6.5) and vapor-liquid equilibrium calculation data of ethanol-water mixture at various vacuum pressures.

RESULTS AND DISCUSSION

Batch fermentation experiments under vacuum pressures: The experimental data of cell dry mass, ethanol and glucose concentration are shown in Fig. 2a and b. Figure 2 show four experimental runs of the initial glucose concentrations of 263.8, 256.5, 220 and 263.2 g L^{-1} at pressure of 760, 100, 65 and 47 mmHg, respectively.

Figure 2a and b show the time course of cell dried mass, glucose consumption and ethanol formation of the four experiments. The reducing pressure does not affect the survival ability of yeast. Although, oxygen/air was not supplied to the fermentation medium, the baker's yeast survived and converted glucose to ethanol for both conditions: at atmospheric and vacuum pressures. The experimental results revealed the dry mass increased slightly at the lag phase, rapidly at the exponential growth phase and entered the stationary phase after 40 h. The weight of biomass increased with increasing pressure. However, after prolong run, the biomass decreases for atmospheric pressure. This trend shows product inhibition. The highest value of the dry mass is 5.01 at 256 g L^{-1} initial glucose concentration and 100 mmHg. At low-pressure conditions, the ethanol concentration was approximately 50 g L^{-1} while the ethanol concentration at atmospheric pressure could reach 116.6 g L^{-1}. These results are explained because when fermentation system operated at vacuum pressure, ethanol evaporated continuously, leaving a fermentation broth concentration lower than that at atmospheric pressure. In case of the system operated at atmospheric pressure, ethanol was not withdrawn so the broth ethanol concentration is higher. Results in Fig. 2b also showed that the cell dry mass in the fermentor remained lower at 760 mmHg when compare to those values at low pressures. In the case of low pressure conditions, the cell dry mass continues to show an upward tendency. However, the fermentation time was not enough to conclude that glucose could be converted completely. And also, the pressure should be reduced further to take ethanol out of fermentation liquid.

For purpose of comparison among four experimental data, an equation for calculation of productivity is presented as follows:

$$\text{Productivity} = \frac{\text{Total amount ethanol produced (g)}}{\text{Volume of fermenter (L)} \times \text{Fermentation time (h)}} \quad (12)$$

The total amount of ethanol produced is the sum of the ethanol collected in the cold trap and the ethanol remaining inside the fermentation vessel.

The comparison of the productivity for four experimental data are shown in Fig. 3.

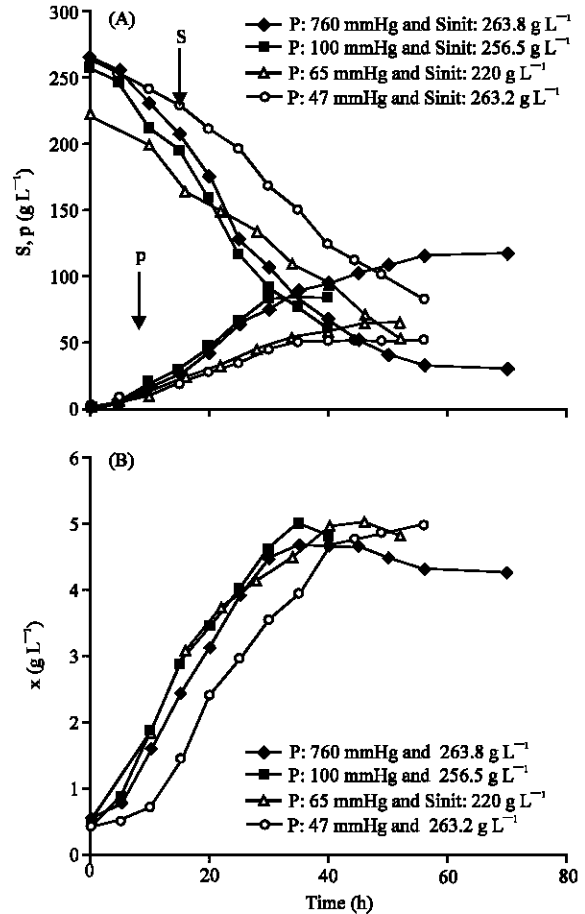

Fig. 2: Time courses of (A) glucose utilization (s), ethanol formation (p) and (B) cell mass formation (x) at various initial glucose concentrations and vacuum pressures

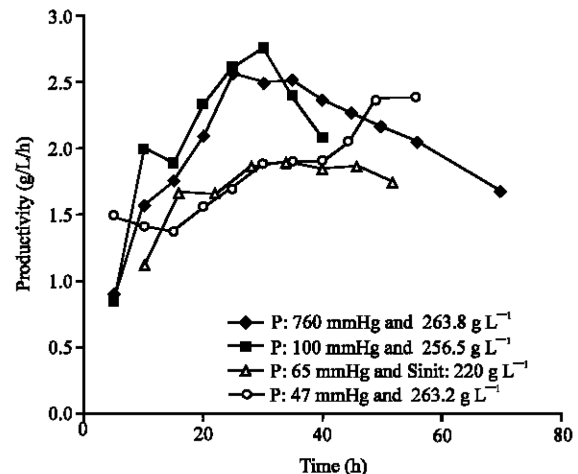

Fig. 3: Results of productivity of batch fermentation at various pressures

The results in Fig. 3 show the difference among four values of productivity when fermentation processes were operated at various pressures. The maximum productivity reached 2.67 g/L/h at 256.5 g L^{-1} of initial glucose concentration and 100 mmHg. Figure 3 showed that at high pressure, the productivity is higher at the start compared to the run under high vacuum, however when the ethanol concentration in the fermentation broth is higher, the productivity started to decline. Compared to the runs under high vacuum, the productivity was initially low and then it continuously increased. Based on Fig. 3, productivity at atmospheric pressure started to decline at around 30 h when the ethanol concentration is around 75 g L^{-1}, while high vacuum runs continued to increase even after a long period. This indicated that the product inhibition that occurred at higher pressures, did not occur at high vacuum runs. The curves in Fig. 3 also indicated that at atmospheric pressure, the productivity was reduced when fermentation time increased. On the other side, the productivity at low pressure had increasing trend when the fermentation time increased. Although, the concentration of ethanol is high in fermentation broth at atmospheric pressure, compared to that under vacuum, the productivity is higher under vacuum than under atmospheric condition.

Another advantage of the fermentation process under vacuum pressure came from the high ethanol concentration of the outlet in cold-trap. In this study, liquid nitrogen was used as coolant to trap the evaporated ethanol-water mixture and byproduct carbon dioxide. The concentration of ethanol in outlet was recorded and shown in Fig. 4.

The results in Fig. 4 show that the combination of fermentor and vacuum system can remove a significant amount of very high ethanol concentration in vapor phase from the fermentation liquid. At the end of fermentation period, these ethanol concentrations can reach a maximum of approximately 50% by weight. At this high ethanol product concentration, it will require low energy to convert to absolute ethanol than the usual atmospheric fermentation product of up to 15% (volume) ethanol only.

Results of simulation: The mechanism and behavior of fermentation under vacuum can be described by using kinetic models. The system of equations includes equation for cell mass balance (Eq. 4), ethanol balance (Eq. 7) and glucose balance (Eq. 9), Together with specific growth rate (Eq. 10) and specific production rate (Eq. 11). The set of parameters obtained from the batch experimental data at atmospheric pressure is shown in Table 1.

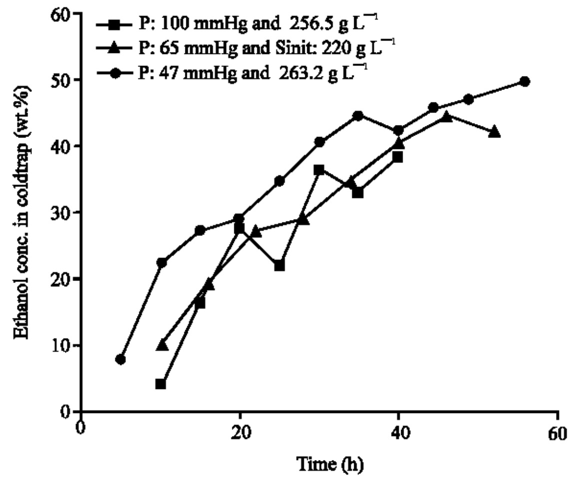

Fig. 4: Ethanol concentrations in cold-trap at various vacuum pressures

Table 1: Experimental values of batch kinetic parameters (μ_m, K_s, K_i, P_m, ν_m, K_s', K_i', P_m', $Y_{p/s}$) from Nguyen *et al.* (2009)

Parametrs	Values
μ_m (h^{-1})	0.354
P_m (g L^{-1})	96.167
K_s (g L^{-1})	6.704
K_i (g L^{-1})	109.470
$Y_{p/s}$	0.483
ν_m (1/h)	4.092
K_s'(g L^{-1})	102.780
K_i'(g L^{-1})	23.886
P_m' (g L^{-1})	401.300

Table 2: The set of kinetic parameters at various pressures

	Pressure (mmHg)			
Parameters	760	100	65	47
μ_{max} (h^{-1})	0.354	0.3500	0.3466	0.3079
P_m (g L^{-1})	96.167	81.8870	60.3440	57.1190
K_s (g L^{-1})	6.704	6.3054	8.9310	9.5227
K_i (g L^{-1})	109.470	134.0500	97.9600	118.8000
$Y_{p/s}$	0.483	0.4720	0.4638	0.4108
ν_{max} (h^{-1})	4.092	3.9720	2.3620	2.2839
P_m' (g L^{-1})	102.780	88.2450	64.1340	53.3760
K_s' (g L^{-1})	23.886	22.3830	27.1840	29.6570
K_i' (g L^{-1})	401.300	283.0500	523.4800	476.5100

The effect of vacuum pressure on the ethanol fermentation process by baker's yeast was studied by a set of kinetic parameters related to cell dried mass, ethanol production and glucose utilization. Using SSE technique, the values of the kinetic parameters estimated from the experimental data at 33°C and various pressures from 760 to 47 mmHg were obtained. Table 2 shows the evident effect of various vacuum pressures on the fermentation process.

The results in Table 2 show that the maximum specific growth rate (μ_{max}), maximum specific production rate (ν_{max}) and the threshold ethanol concentrations (P_m and P_m') reduced as system pressure reduced. These behaviors are

Table 3: Statistical results of R^2 of experimental data at various pressures

R^2 (mmHg)	X	P	S
760	0.9712	0.9590	0.9712
100	0.9915	0.9919	0.9915
65	0.9277	0.9277	0.9277
47	0.9167	0.9860	0.9167

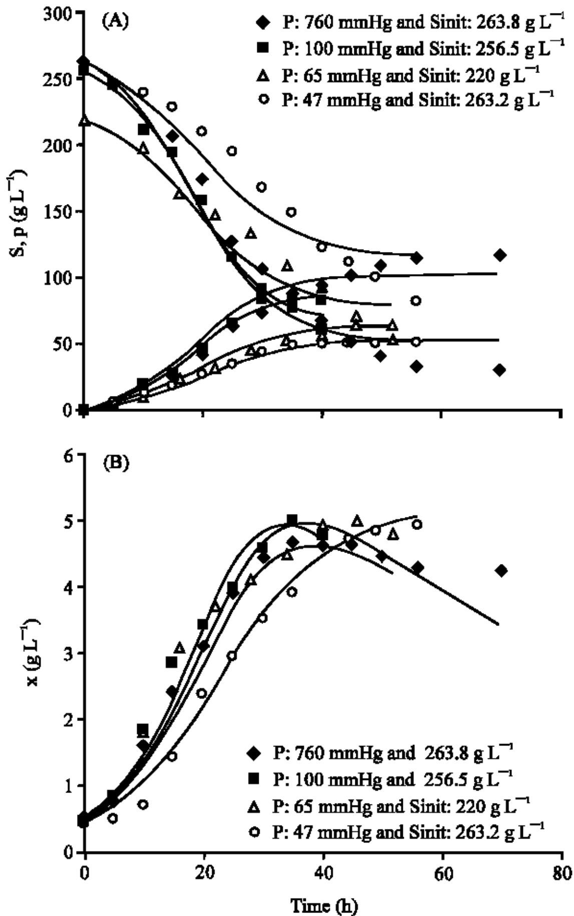

Fig. 5: Experimental results and simulation of (A) glucose (s), ethanol concentrations (p) and (B) cell dried mass at various pressures; lines correspond to model simulation while dots correspond to experimental data

explained by the accumulation of cells in fermentation broth and removal of ethanol during fermentation period. The other parameters were not changed so much so that their values are considered to remain as system pressure reduced. These results show that the substrate inhibition phenomenon still remained in fermentation broth even at reduced system pressure. But its effect is not very significant under vacuum compared to that of under atmospheric pressure.

The accuracy of kinetic parameters was ensured by the comparison between estimated and experimental data. The statistical results of R-square of cell dried mass concentration, ethanol concentration and glucose concentration are shown in Table 3.

The results in Table 3 show a good agreement between estimated and experimental data of the time course of cell dried mass, ethanol formation and glucose utilization. These results also guarantee the above calculation method. The experimental results and simulation of the cell dry mass, glucose concentration and ethanol concentration at various pressures are demonstrated in Fig. 5a and b. The estimations gave an adequate fit to the experimental data for all cell dry mass, glucose and ethanol concentrations. This indicated that the proposed kinetic model and sets of parameters were valid for data interpretation.

CONCLUSIONS

A batch ethanol fermentation processes at various system pressures were investigated in this study. The baker's yeast used as inoculum can survive and convert glucose to ethanol at anaerobic condition and vacuum pressure. Using the combination of fermentation process and vacuum system, a very high ethanol concentration can be collected at the outlet. The productivity of ethanol at vacuum pressure increased when compared to the productivity at atmospheric pressure. The data showed that product inhibition occurred at atmospheric condition as shown by declining of productivity when the ethanol concentration in the broth became significant after running for a long time.

While, the inhibition effect is significantly reduced under vacuum as shown by increasing productivity even after prolong runs due to low concentration of ethanol in the broth caused by continuous removal of ethanol through evaporation under vacuum.

The results from kinetic rate equations and parameters indicated that the kinetic model developed from batch experimental data at atmospheric pressure can apply for low pressure condition. The results collected from these batch experiments opened new insights on the study of continuous alcohol fermentation at low pressure.

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