Chapter 4
RESULTS AND DISCUSSION

4.1 Fermentation

Batch fermentation experiments in shake flasks for ethanol production have been carried out in duplicate for 72 hours with *Saccharomyces cerevisiae* M30 culture using molasses as substrate. First, the experiments are performed at the initial reducing sugar concentration varied from 3 to 25 %w/v to determine the effect of initial sugar concentration and the operating temperature is maintained at 33°C. The results of initial sugar concentration are shown in Figure 4.1 a, b, and c. At the high initial substrate concentration of 25%w/v, a significant inhibition of cell growth can be observed, whereas at the lower initial substrate concentration values from 5 to 22%w/v the growth rates are greater. The experiments point up that the values of maximum specific growth rate of the cell decreases appreciably and maximum specific production rate increases considerably with the increasing of initial sugar concentration. The results relate to the observation of initial sugar concentration effect on yeast metabolism. It is well known that an important effect of initial reducing sugar above 30 g/L is metabolize repression of oxidation pathway leading to switch from the oxidation of glucose for cell growth to ethanol production by fermentation [Birol, *et. al.*, 1998]. The experiment shows that 22%w/v reducing sugar concentration is the maximum point of both cell growth and ethanol production.

Second, the experimental studies are performed at 22%w/v of initial reducing sugar concentration and the operating temperatures is controlled at five different values; 30, 33, 35, 38 and 42°C. Experimental results reveal the biomass enters a stationary phase between 12 and 18 hours after inoculation in all operating temperatures as illustrated in Figure 4.2 a. At the operating temperature 33°C, cell growth and ethanol productivity increase compared to 30°C and slightly decreased at 35°C. Cell growth and ethanol productivity are declined significantly at the operating temperature 38°C and 42°C. It can be concluded that operating temperature directly affects cell growth. The observed optimal temperature for cell growth and ethanol productivity is at

33°C. The existed substrate concentration depends on its conversion to cell growth and ethanol production. At 33°C operating temperature, substrate is nearly depleted and the residual sugar which cells are unable to consume still remains less than 50 g/L (Figure 4.2 b). The maximum ethanol concentration is obtained after 35 hours of the fermentation at 30°C as shown in Figure 4.2 c which relevant with Torija's experiment [Torija, *et. al.*, 2003]. His experiment showed that alcohol yield was higher at low temperatures. Due to the high operating temperature at 38°C and 42°C, ethanol concentration at the end of fermentation is slightly deteriorated from its evaporation.

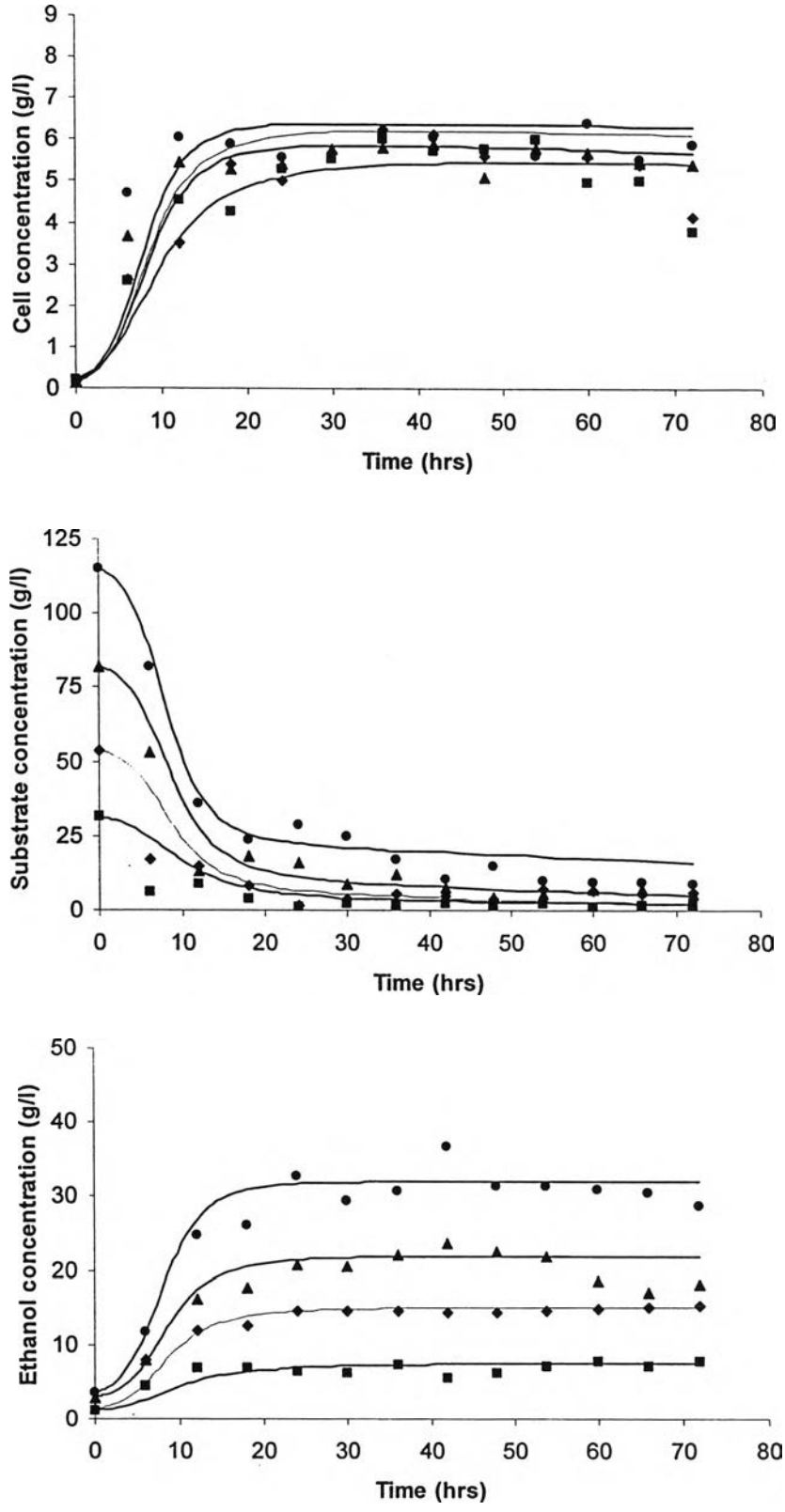


Figure 4.1 (a) Experimental results and simulation of cell, substrate and product concentrations at different initial substrate concentrations; Lines correspond to model simulation while dots correspond to experimental data. (■ 3%, ◆ 5%, ▲ 8%, ● 11%)

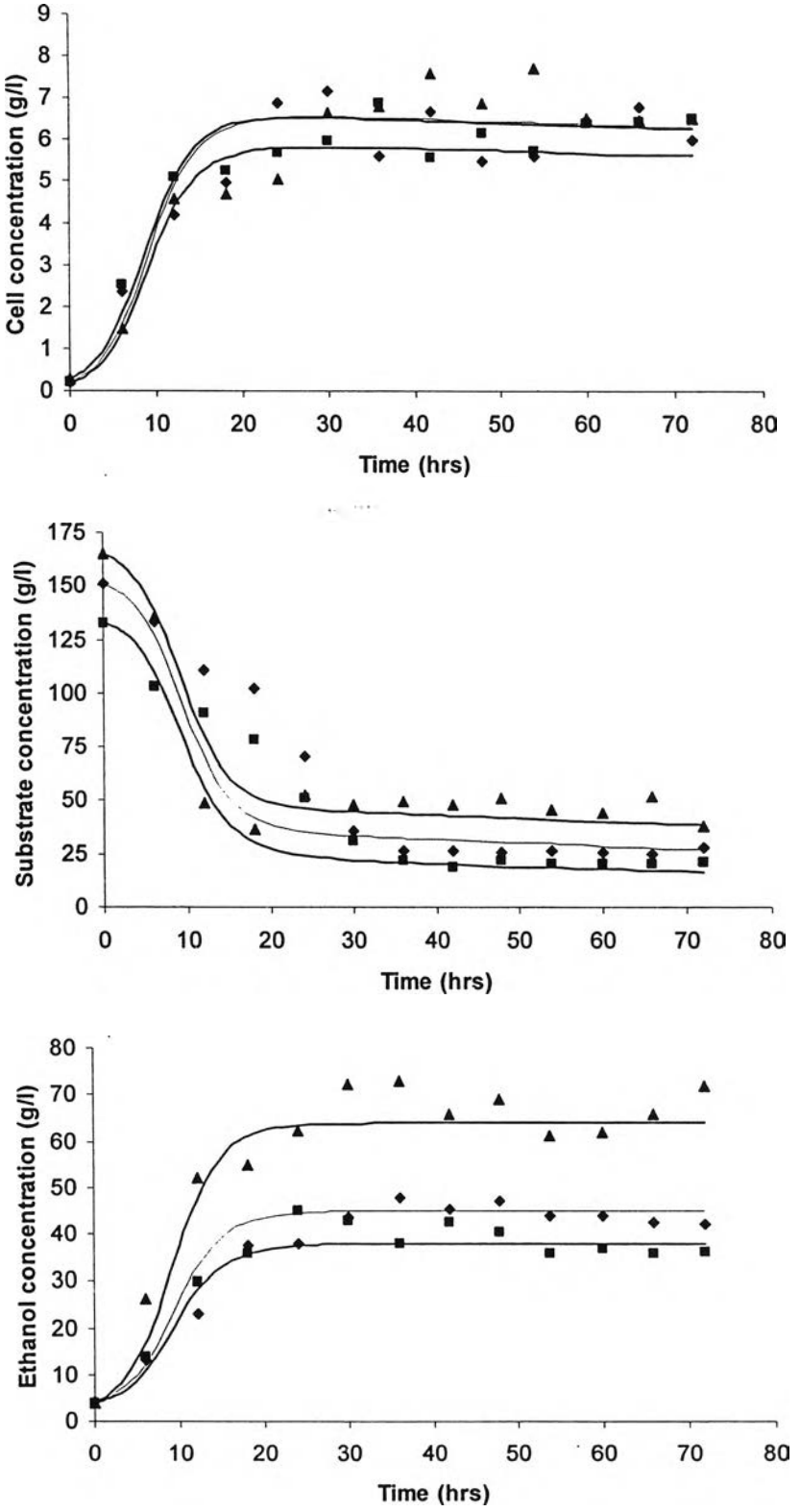


Figure 4.1 (b) Experimental results and simulation of cell, substrate and product concentrations at different initial substrate concentrations; Lines correspond to model simulation while dots correspond to experimental data. (■ 13%, ◆ 15%, ▲ 17%)

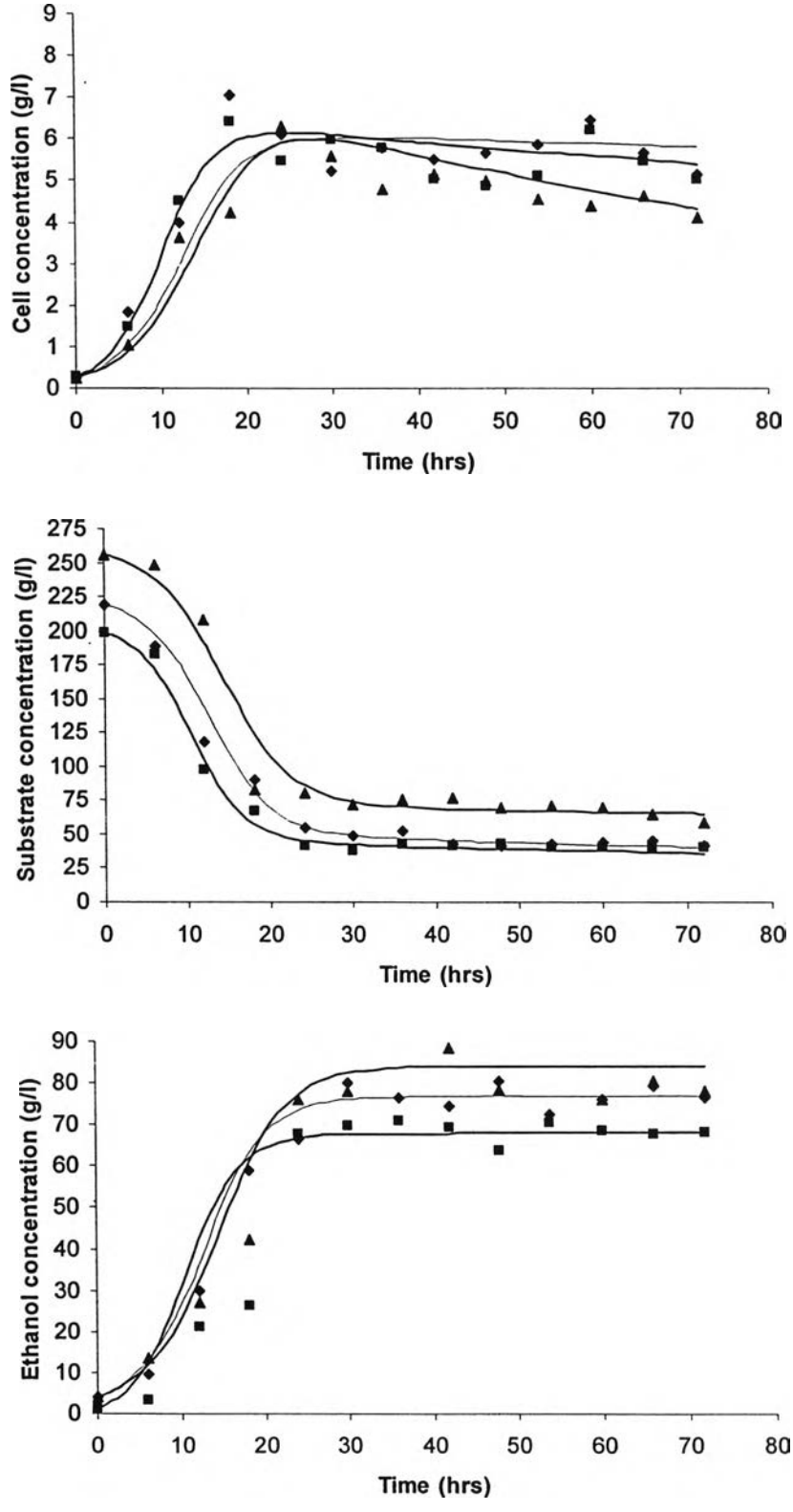


Figure 4.1 (c) Experimental results and simulation of cell, substrate and product concentrations at different initial substrate concentrations; Lines correspond to model simulation while dots correspond to experimental data. (■ 20%, ◆ 22%, ▲ 25%)

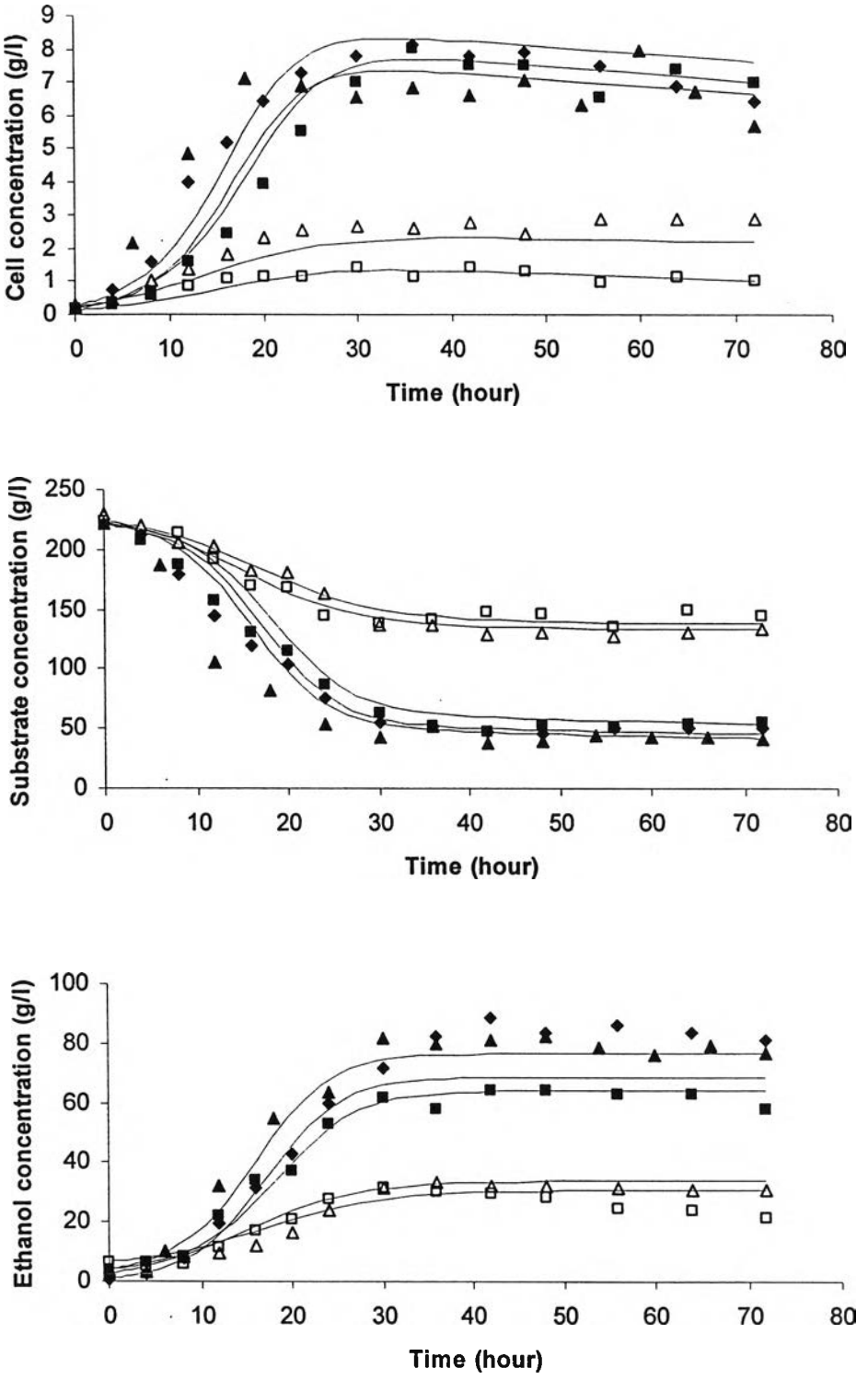


Figure 4.2 Experimental results and simulation of cell, substrate and product concentrations at different operating temperatures; Lines correspond to model simulation while dots correspond to experimental data.

(◆ 30°C, ▲ 33°C, ■ 35°C, △ 38°C, and □ 42°C)

4.2 Mathematical model

The fermentation data from ethanol batch fermentation with varying initial substrate concentrations are used to estimate the initial guesses of kinetic parameters. The maximum specific growth rate (μ_m), saturation constant of substrate (K_S), maximum specific production rate (ν_m) and saturation constant of product (K_{SP}) are calculated by using the initial rate method. The growth yield is assumed to be 0.5 g/g at 33°C operating temperature for *Saccharomyces cerevisiae* [Edwin, 1990], thus the product yield can be calculated by the overall mass balance of substrates. The assumption is comparable with the Najafpour's experiment which was found that the maximum yield of biomass on substrate was 50.8% [Najafpour, *et. al.*, 2004]. These initial guesses of the parameter values are used in the first loop of numerical method solving.

The modified Monod kinetics is used in this study [Sainz, *et. al.*, 2003]. The cell balance counted with death rate and cell maintenance, the ethanol and the substrate balance are then set for the description of growth and fermentation. Reducing sugar from molasses is assigned as the sole limiting substrate of the fermentation. The effects of initial substrate concentrations, C_{S0}, on the kinetic parameters are investigated. The mathematical model of the microbiological ethanol synthesis can be written as follows:

$$\mu C_X = \frac{dC_X}{dt}$$

$$= \frac{\mu_m(C_{S0})C_S}{K_S(C_{S0}) + C_S + \frac{C_S^{\,2}}{K_{SS}(C_{S0})}}\left(1 - \frac{C_P}{P_m(C_{S0})}\right)C_X - K_d(C_{S0})C_X - K_{CM}(C_{S0})C_X \qquad \text{------- (4.1)}$$

$$\nu C_X = \frac{dC_P}{dt}$$

$$= \frac{\nu_m(C_{S0})C_S}{K_{SP}(C_{S0}) + C_S + \frac{C_S^{\,2}}{K_{SSP}(C_{S0})}}\left(1 - \frac{C_P}{P_{mP}(C_{S0})}\right)C_X \qquad \text{------- (4.2)}$$

The inhibitory effects of high initial sugar concentration are also observed in terms of substrate growth inhibition factor ($1/K_{SS}$) and substrate

production inhibition factor ($1/K_{SSP}$) as shown in equation 4.1 and 4.2, which increase considerably with the increasing of initial sugar concentration. The effect of corn stover hydrolysate on the maximum specific growth rate of 24 selected yeast strains in ethanol fermentation was reported by Evans in 2002 [Evans, *et. al.*, 2002].

The initial guess of kinetic parameters from the batch experiments calculated by using the initial rate method are then re-calculated by MATLAB programming until they reach minimum error with nonnegative value constrains that shown in Table 4.1.

Table 4.1 Kinetic parameters at different substrate concentrations.

Substrate concentration [g/L]	μ_m [h^{-1}]	K_S [g/L]	K_{SS} [g/L]	P_{mx} [g/L]	K_d [h^{-1}]
30	1.0771	45.48	22500	7.40	7.50E-04
50	0.9562	45.31	20500	14.50	7.50E-04
80	0.8023	43.27	19000	22.50	9.50E-04
110	0.7034	40.92	13300	31.60	9.05E-04
170	0.5021	37.73	1250	60.00	8.50E-04
200	0.4305	37.26	858	67.81	8.20E-04
220	0.3902	29.52	595	76.74	8.90E-04
250	0.3768	29.15	573	79.75	8.25E-04

Substrate concentration [g/L]	ν_m [h^{-1}]	K_{SP} [g/L]	K_{SSP} [g/L]	P_{mp} [g/L]	K_{CM} [h^{-1}]	$Y_{P/S}$ [-]
30	1.2150	47.51	22500	7.40	0.0060	0.351
50	2.1350	39.48	22000	14.50	0.0100	0.359
80	2.4245	38.00	21800	22.50	0.0150	0.364
110	2.8535	29.86	15000	31.60	0.0172	0.383
170	3.4860	9.08	1110	60.00	0.0226	0.481
200	3.7560	7.75	868	67.91	0.0241	0.468
220	3.8704	4.97	833	76.80	0.0293	0.457
250	3.8377	5.38	700	82.50	0.0215	0.452

The effects of initial sugar concentration on kinetic parameters are investigated and the functions can be given by polynomial equation. For example, the equations are shown in equation 4.3 and 4.4 and Figure 4.3.

$$\mu_m = 1.1674\times10^{-5} C_{S0}^{\ 2} - 6.5176\times10^{-3} C_{S0} + 1.2711 \qquad \text{-------- (4.3)}$$

$$\nu_m = -5.0825\times10^{-5} C_{S0}^{\ 2} + 2.5010\times10^{-2} C_{S0} + 0.8127 \qquad \text{-------- (4.4)}$$

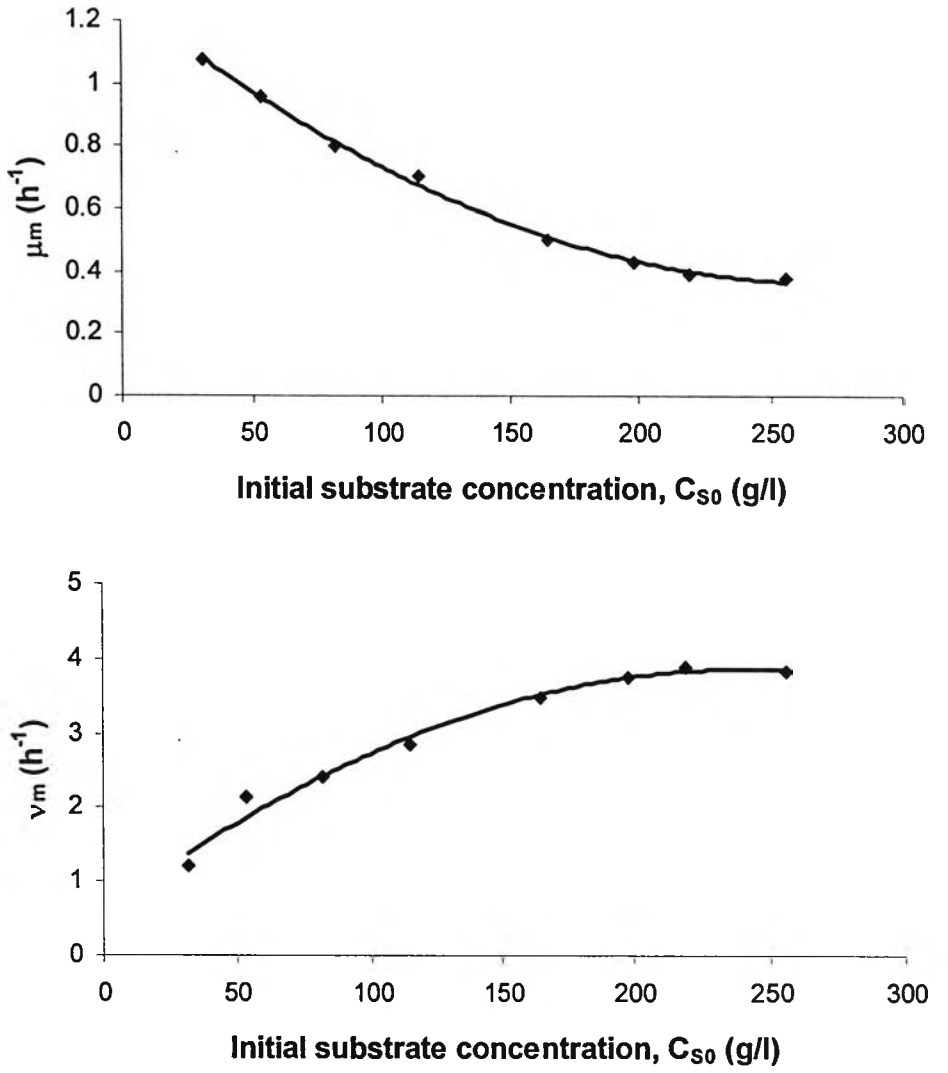


Figure 4.3 The relationships between: (a) the initial sugar concentration (C_{S0}) and the maximum specific growth rate (μ_m); (b) the initial sugar concentration (C_{S0}) and the maximum production rate (ν_m)

The production yields estimated in this study is 0.35-0.45, which is relevant to that from other reports [Ghaly, *et. al.*, 1997]; and it was also relate with Najafpour's experiment [Najafpour, *et. al.*, 2004]. The functions of initial substrate concentration on the kinetic parameters can be given by polynomial equations similar to the functions proposed for a kinetic model for beer production by Andrés-Toro's in 1998 [Andrés-Toro, *et. al.*, 1998].

Subsequently, the superposition temperature term is applied to the prior key model parameters.

$$\mu_m, K_S, P_{mx}, \nu_m, P_{mp}, Y_{X/S}, \text{and } Y_{P/S} = f(C_{S0}, T) \qquad \text{------- (4.3)}$$

$$\frac{\text{Parameter}}{\text{Parameter at reference temperatu re}} = \gamma_i = \frac{a_i e^{-\frac{b_i}{T}}}{1 + c_i e^{-\frac{d_i}{T}}} \qquad \text{------- (4.4)}$$

where γ is the ratio of specie i parameter between its temperature and reference temperature while a, b, c, and d are the coefficients of specie i.

The example of the ratio is shown below.

$$\frac{\mu_m}{\mu_m^o} = \gamma_{\mu_m} = \frac{a_{\mu_m} e^{-\frac{b_{\mu_m}}{T}}}{1 + c_{\mu_m} e^{-\frac{d_{\mu_m}}{T}}} \qquad \text{------- (4.5)}$$

The growth, production and yield key parameters are carried out in both initial sugar concentration and temperature term with best fit result as shown in equation 4.3 and 4.4. The reference value of each parameter is set with the 22%w/v reducing sugar concentration at 33°C operating temperature.

Finally, the parameters compose the effects of both initial substrate and ethanol concentration and temperature. The result of parameter fitting in exponential from are shown in Table 4.2 and Figure 4.4.

Table 4.2 Kinetic parameters in term of ratio between their temperature value and reference temperature value

T (K)	γμm	γKs	γKd	γνm	γPmx=γPmp	γYxs=γYps
303	0.92	1.0	1.1	0.92	1.08	1.10
306	1.00	1.0	1.0	1.00	1.00	1.00
308	1.10	2.0	2.0	1.00	0.90	0.95
311	1.30	5.0	5.0	1.10	0.69	0.75
315	1.50	10.0	10.0	1.43	0.40	0.65

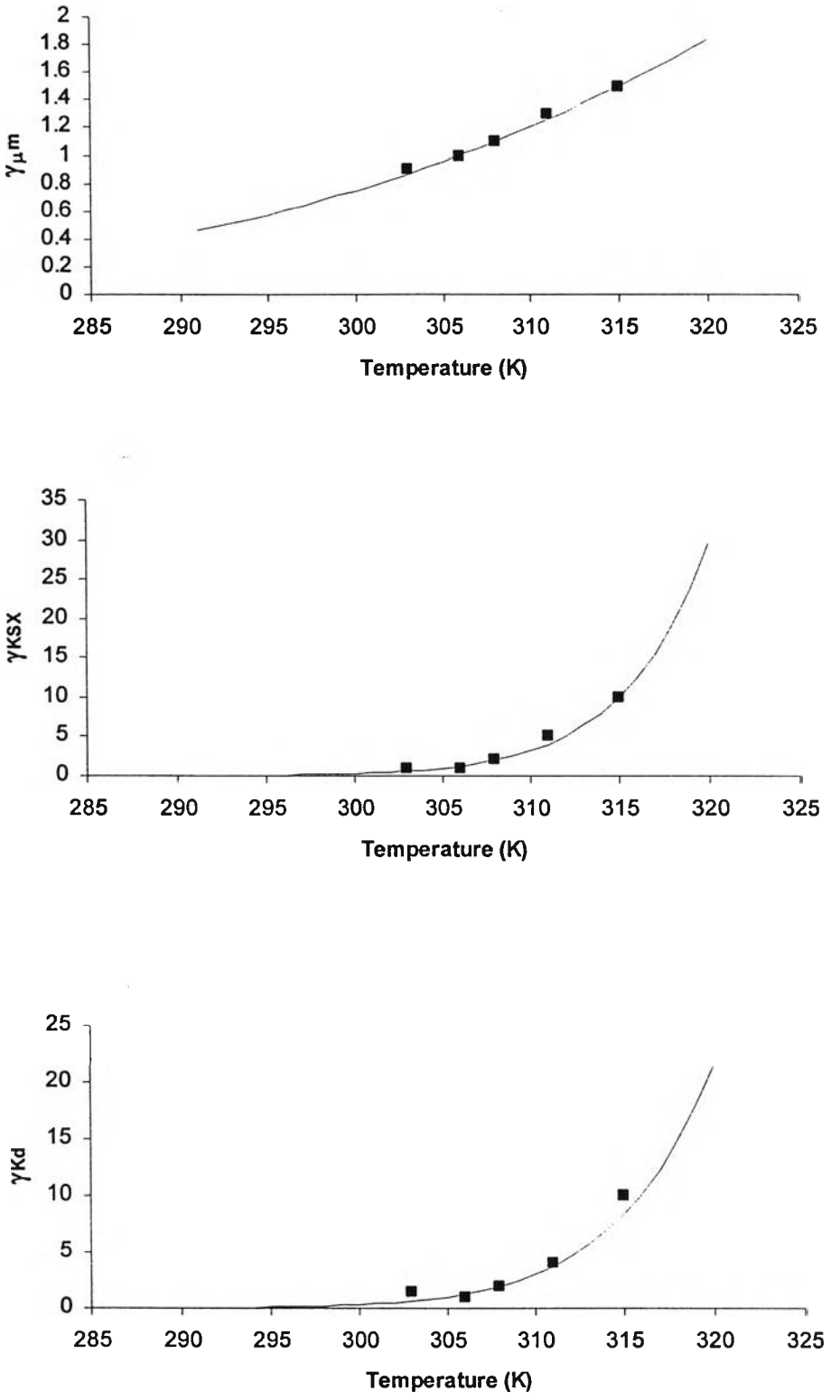
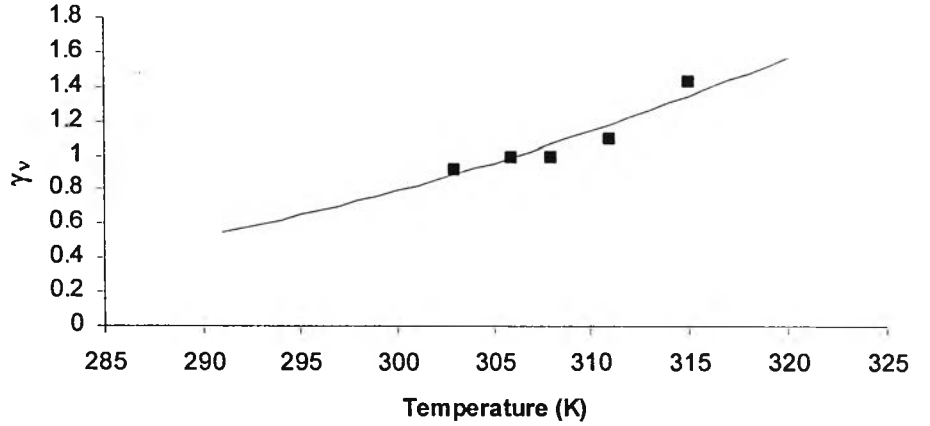
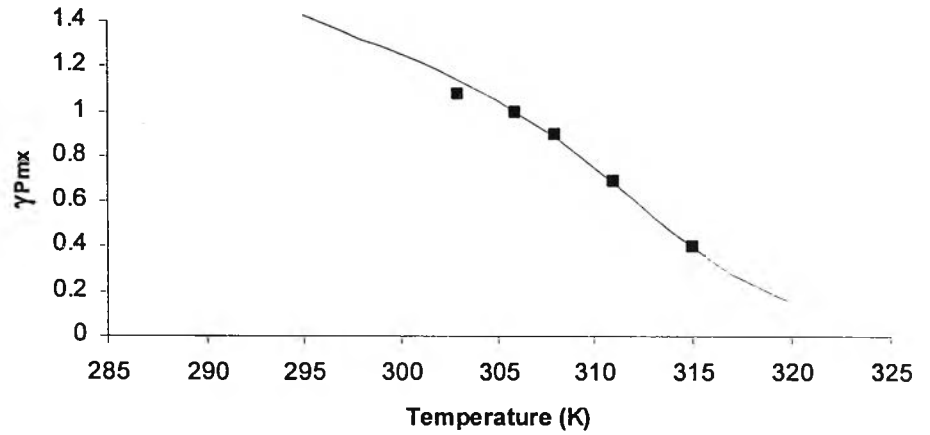


Figure 4.4 (a) Ratio of each parameter on its reference temperature parameter (33°C); Lines correspond to the simulation of superposition temperature term while dots correspond to the parameter ratio obtained from the experiments.





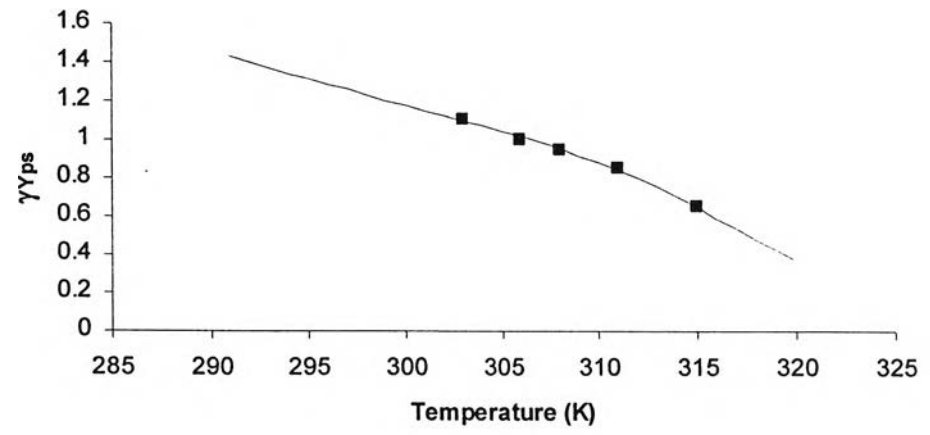


Figure 4.4 (b) Ratio of each parameter on its reference temperature parameter (33°C); Lines correspond to the simulation of superposition temperature term while dots correspond to the parameter ratio obtained from the experiments.

Figure 4.4 shows the correlation between key parameters and temperature. The influence of temperature on cell growth and cell activity can be explained by the superposition of activation and deactivation effects. At high temperature, a fast inactivation in term of cell death rate is accelerated. As a result of both an optimum temperature for growth of cell exists (Figure 4.5). After it reaches maximum kinetic, the effect of temperature on the cell death parameter is superior and more effectiveness than the reproduction parameters. Both substrate yields, growth and production, are function in superposition from which is responding from cell generating and ethanol conversion. Dependency of the initial specific growth rate (μ') at 22%w/v initial sugar concentration is shown in Figure 4.5. The result was similar to other micro organism experiment, *Klebsiella pneumoniae* [Esner, *et al.*, 1980] and *Pachysolen tannophilus* [Sánchez, 2003]. Figure 4.2 represents the predicted and experimental data of cell, substrate and ethanol concentrations at different operating temperature. The simulation reveals that the mathematical model developed in this study makes a good agreement to the experiment results.

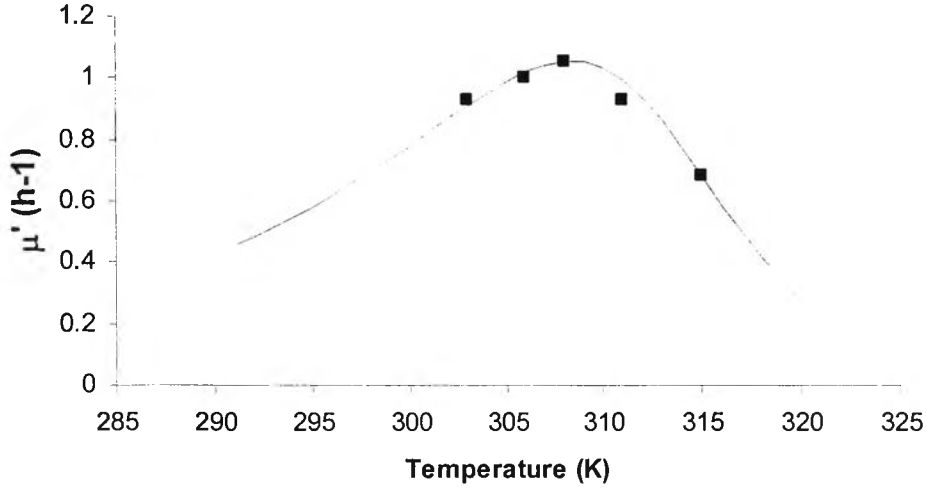


Figure 4.5 Dependency of initial specific growth rate at 22%w/v initial sugar concentration of *Saccharomyces cerevisiae* M30 on the temperature. Lines correspond to the simulation of superposition temperature term while dots correspond to the maximum specific growth rate obtained from the experiments.