

Full Length Research Paper

Mathematical modeling of fructose production by immobilised glucose isomerase as a function of temperature and pH variations

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Accepted 25 February, 2011

Production of fructose from glucose isomerisation process using commercial immobilized glucose isomerase (IGI) was conducted in a batch type of stirred tank bioreactor. A mathematical model was developed to describe the effect of temperature and pH on the kinetic parameters of fructose production. Modified Santos model known as MM3 was used to describe this phenomenon. The influence of temperature and pH was investigated and quantified. The results showed that, even though the highest R^2 was at 70 °C but based on the IAE of 0.843 and ISE of 0.978, it proved that for enzymatic reaction, it should be carried out below 65 °C. Effect of pH for various models have shown that, the IAE and ISE were less than one whereas the R^2 were greater than 0.95. This indicates that the model, MM3 is acceptable.

Key words: Batch reactor, fructose, glucose isomerisation, mathematical modeling, pH, temperature.

INTRODUCTION

A common method of modeling is where a set of mathematical equations are programmed and simulated by using software such as Matlab, C++, Labview and others. The other type of modeling is called empirical modeling where a model is developed based on the data obtained from experiments. Developments of effective modeling require assumptions which are based on previous researches or from preliminary experiments. Apart from that, development of modeling from mathematical models can be quite tedious and difficult especially if it involves too

many mathematical equations.

In this case study for enzymatic reaction, which occurs at very fast reaction rate, an intermediate approach was taken in order to counter balance between advantages and disadvantages of modeling. Empirical modeling suggested for this work would be based on the data obtained from experimental work. The parameters obtained from the experiment such as maximum velocity, V_m , and Michaelis constant, K_m , would be used in the modeling. Other operating parameters in the experimental works, such as temperature, pH and concentration of substrate, amount of enzymes, optimum agitation speed and feed flow rate would be introduced in the model in order to imitate the real situation.

Glucose isomerisation process is an example of enzymatic reversible reaction which involved only one substrate which is glucose and a product, namely fructose. An enzyme known as immobilized glucose isomerase (IGI) from *Streptomyces murinus* was used in this research. This type of process is the most successful industrial application in immobilized enzyme technology. Most researchers obtained the data for simulation from experi-

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Abbreviations: IGI, Immobilized glucose isomerase; AAE, average absolute error; IAE, integral absolute error; ISE, integral square error; STR, stirred tank reactor; MM3, modified Santos-MM model; MM3, proposed model; MM, Michelis-Menten; SMM, Santos-Michelis-Menten; HPLC, high performance liquid chromatography; ACN, deionised water and acetonitrile.

mental works where some of them obtained from industrial data (Illanes et al., 1992).

Researchers, in developing the model, made quite similar assumptions as those made by Asif and Abaseed (1998) which are:

- The system is isothermal.
- Effective diffusion coefficient is constant and independent of concentration.
- Uniform enzyme activity throughout the particle.
- The agitation is strong enough to ensure perfect mixing.

Therefore, in this present study, an optimum amount of enzyme, temperature, pH and agitation speeds for the reaction are considered while modeling for the process under batch and continuous operations. A comprehensive methodology for the design of reactor using immobilized enzymes as catalyst was also presented by Illanes et al. (1992), but the source of commercial IGI was obtained from Miles (1987) and control of pH and temperature was not mentioned.

In this study, the model development consists of diffusion and reaction mechanisms which are:

- Reaction kinetics focusing on temperature and pH effect for enzyme.
- Mass transfer for solid phase and liquid phase.
- Reactor performance.

With the constraint of temperature and pH of the reaction, parameters for the proposed model were compared with those from experimental works and from previous study. The proposed model (MM3) was compared with the Michaelis-Menten (MM) model and Santos-Michaelis-Menten model (SMM).

MATERIALS AND METHODS

The chemicals used in this study were D-glucose (G), D-fructose (F) and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (R and M Chemical, UK). 12 g of immobilised glucose isomerase (IGI) was used for batch reactor and 6 g of IGI was used for the fixed bed reactor and recycled fixed bed reactor. The immobilised glucose isomerase (IGI) was obtained from *S. murinus*, some brown cylindrical shaped granules of diameter 0.3 to 1.0 mm, length 1.0 to 1.5 mm and activity of 350 IGIU/g (Sweetzyme, Novozymes, Denmark). For the high performance liquid chromatography (HPLC) analysis, deionised water and acetonitrile (ACN) (HPLC grade) are used. Different series of glucose concentrations from 10 to 50% w/v were prepared. All analytical samples were diluted with distilled water and filtered through 0.45 μm Nylon filters prior to the HPLC-analysis.

Determination of kinetic parameters

Enzyme activity, kinetic parameter of the process, initial reaction rate, maximum velocity, V_m , and Michaelis-Menten constant K_m were determined before the reactions were conducted. The method

to determine kinetic parameters, V_m , and Michaelis-Menten constant K_m , was adopted from the pioneering work of Converti and Borghi (1997), where 0.1 M of glucose was diluted to 1 L of solution A, and the solution was adjusted to pH 7. 12 g of IGI were added into the bioreactor. The rehydration of IGI was done at two conditions, rehydrated at cool room (4°C) then at room temperature (27°C). The reaction was done at 55, 60, 65 and 70°C with agitation speed of 150 rpm. Five ml of samples was taken for every 2 min for the first 10 min, followed by every 10 min for the next 2 h of the reaction. Each sample was boiled for 15 min to deactivate the enzymes (Salehi et al., 2005; Lee and Hong, 2000) and cooled down to room temperatures. For the analysis of glucose and fructose, HPLC was used with UV detector at 195 nm (Rahman et al., 2008).

Experimental set-up for glucose isomerisation

As shown in Figure 1, the reactor system consists of a 2 litre double-jacketed reactor, made from Borosilicate glass (3.3 DN 120 043943). The reactor was connected to a water bath (Huber) and is equipped with propeller type impeller driven by a motor (Heildoph, Germany with RZR323 control). The motor of the agitator can be manually adjusted, ranging from 50 to 2000 rpm the function of the water bath was to control the jacketed reactor at the required temperature. The feed consists of 1 L of solution containing 18 g of glucose and 1 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (that is, 0.1 M glucose and 1 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in a distilled water). All the experiments were conducted at constant agitation speed of 150 rpm. The enzyme was rehydrated with distilled water for 24 h in the cold room before being added to the reactor. The reactions were carried out at different temperatures (55, 60, 65 and 70°C) and different pH (4, 6, 8 and 9) in a non-buffer solution. Samples were withdrawn every 10 min for analysis.

Model development

The model development is based on the diffusion-reaction system, as it involved the heterogeneous system that is in solid-liquid phase. The solid phase is referred to as the immobilized glucose isomerase and the glucose solution as the liquid phase. Most of the researchers used the basic Michaelis-Menten equation for the reaction mechanism but with some modification, such as comparing between α and β D-glucose (Lee and Hong, 2000), optimizing reactor design (Salehi et al., 2004; Illanes et al., 1992; Racki et al., 1991; Asif and Abasaeed, 1998) and studies of the thermodynamics of the process by Converti and Borghi (1997). According to Illanes et al. (1992), mechanism of enzymes are similar catalysts, but very specific for certain reactions. This model is focused on the temperature and pH effects on an enzyme, since the thermal inactivation of an enzyme is usually the limiting factor for reactor performance.

The model in this study will consider the temperature region where an enzyme starts to deactivate, similar with Santos et al. (2007) with further investigation related to the effect of pH, reactor performance and diffusion resistance. Assumptions of this model include:

- Uniform enzyme activity throughout the particle.
- At steady state.
- The overall system is isothermal.
- Effective diffusion coefficient is constant and independent of substrate concentrations.

One of the objectives in this study is to develop the rate equation for the reaction which takes place on the active site on the surface of the enzyme. According to Levenspiel (1999), Fogler (1999) and Bailey and Ollis (1986), three steps are expected to occur

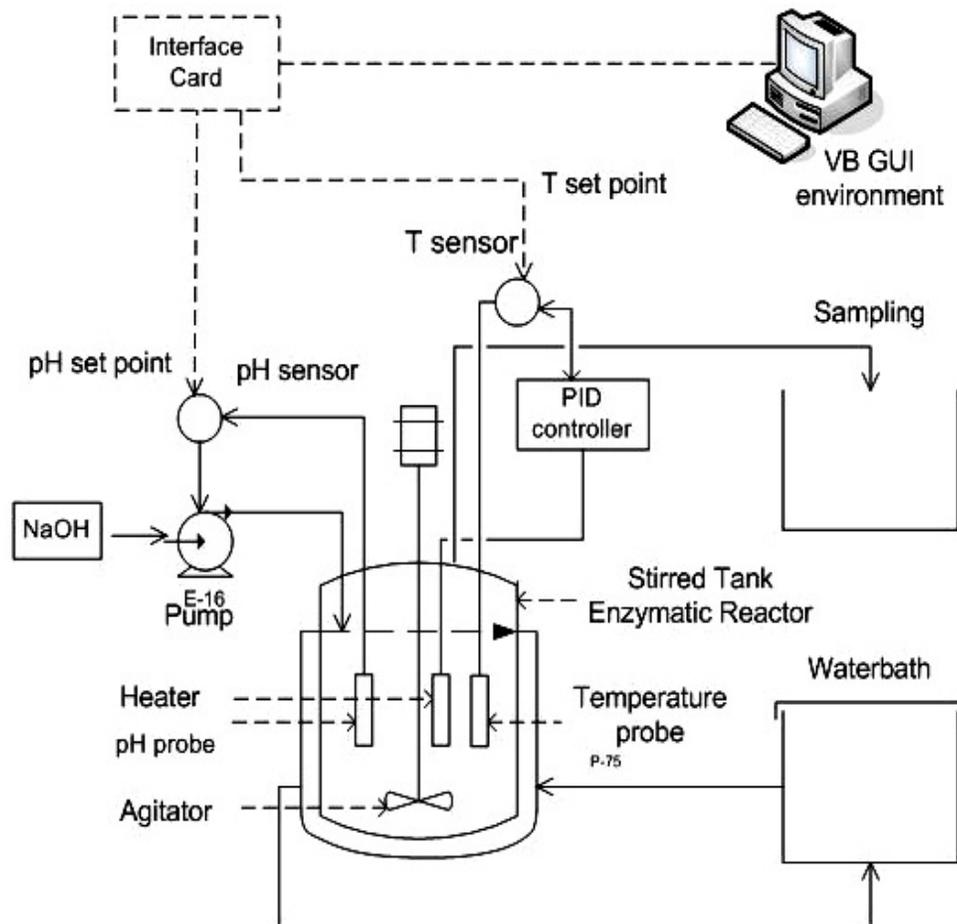


Figure 1. Schematic diagram of batch reactor for glucose isomerisation process.

successively at the surface. The first step is the adsorption of substrate onto the enzyme surface, followed by the surface reaction of substrate to form product and then desorption of product from the surface, for a one substrate (S) and one product (P) system. The final rate of the reaction could be expressed as below;

$$r = \frac{dP}{dt} = -\frac{dS}{dt} = \frac{V_m S}{K_m + S} \quad (1)$$

Equation 1 follows the Michaelis-Menten kinetics. However Lee et al. (1979) showed that the Michaelis-Menten model is not totally valid in this reaction since the enzyme used was an immobilized enzyme in solid form, mixed with the liquid phase. Thus, the reaction rate for the heterogeneous enzyme reaction system can be modified as;

$$r = \frac{V_m' S^*}{k_m' + S^*} \quad (2)$$

Where S^* is a bulk substrate concentration which is readily measurable, compared to substrate concentration inside the catalyst and V_m' and k_m' are the apparent kinetic constants.

Enzyme inactivation

In general, the literature reported that the kinetic of the reaction is close to a pseudo-first-order reaction and have successfully been proposed for thermal inactivation of both free and immobilized forms of this enzyme (Lee et al., 1976). According to Bailey and Ollis (1986), inactive enzyme E_i and active enzyme E_a can be derived as follows:



Thus the rate of enzyme decay

$$r_d = k_d E_a \quad (4)$$

Where k_d is decay constant. For enzyme decay rate,

$$\frac{dE}{dt} = -k_d E_a \quad (5)$$

So that,

$$\ln \left[\frac{E_a(t)}{E_a(0)} \right] = -k_d t \tag{6}$$

Enzyme deactivation in this model is similar with the terms used by Asif and Abaseed (1998) and Santos et al. (2007), with addition of temperature and decay constant, k_d , into the overall reaction rate, r . Enzymatic activity can be expressed by Equation 7 and the kinetic constant, K , varies with temperature, therefore it can be expressed by an Arrhenius type Equation 8.

$$r = KE \tag{7}$$

$$K = K_0 e^{-\frac{E_a}{RT}} \tag{8}$$

K_0 is the activation constant and significant at the lower temperature. From Equation 6, rearranging,

$$E = E_0 e^{-k_d t} \tag{9}$$

Where k_d , is a denaturation constant and temperature dependent and t , is a time when enzyme deactivate. The enzyme, E , is significant at the highest temperatures, that is at the inactivation process. Applying Arrhenius type equation to k_d , we get,

$$K_d = K_{d0} e^{-\frac{E_d}{RT}} \tag{10}$$

Substituting Equations 8, 9 and 10 into 7 gives the final expression for r as a function of temperature and reaction time,

$$r = [K_0 e^{-\frac{E_a}{RT}}][E_0 e^{-(k_{d0} e^{-\frac{E_d}{RT}})t}] \tag{11}$$

In Equation 11, t , should be very small, ≤ 1 min, since only the initial rates are being considered. Since K_0 and E_0 are constants, they were lumped into a constant nominated v_0 .

Substitute v_0 into Equation 11, to obtain r as follows:

$$r = [v_0 e^{-\frac{E_a}{RT}}][e^{-(k_{d0} e^{-\frac{E_d}{RT}})t}] \tag{12}$$

Nevertheless, r is actually present in the general reversible Michaelis-Menten kinetics, Equation 1. Substituting Equation 12 into Equation 2 leads to Equation 13 which represents the rate of reaction of the process which involved the bulk substrate concentration, S^* , V_x' and k_m' , which are the apparent kinetic constants,

$$r = \frac{[v_0 e^{-\frac{E_a}{RT}}][e^{-(k_{d0} e^{-\frac{E_d}{RT}})t}] S^*}{k_m' + S^*} \tag{13}$$

Equation 13 represents the rate of reaction as a function of temperature and bulk substrate concentration.

Another factor which contributes for enzyme denaturation is the pH of solution. pH is a value that represents the acidity or alkalinity of an aqueous solution (Lewis, 2001). In general form, pH is defined as below;

$$pH = \log_{10} \frac{1}{[H^+]} \tag{14}$$

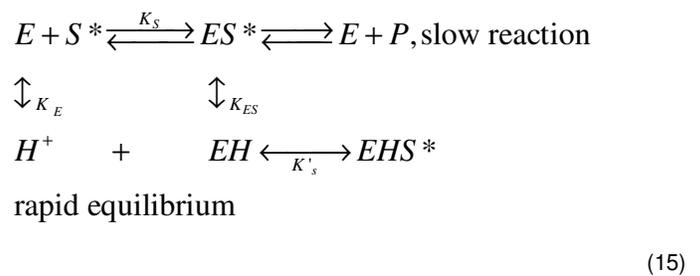
According to Enke (2000) and Blanch and Clark (1997), denaturation of enzyme activity is a function of pH of solution. In this study, pH of solution refers to bulk substrate concentration, S^* , therefore;

pH of S^* = pH of initial substrate = pH of enzyme.

Relationship between rate of reaction as a function of pH of solution could be derived based on three assumptions:

No difference between activity and concentration.
Proton transfer is more rapid than chemical steps.
Rapid equilibrium exists, $[E][S^*] \approx [ES^*]$.

Considering this pathway scheme for binding of substrate to both protonated and unprotonated enzyme;



$E = \text{enzyme}$, $S^* = \text{bulk substrate}$, $ES^* = \text{enzyme substrate complex}$, $P = \text{product}$

$EH = \text{protonated enzyme}$

$EHS^* = \text{protonated enzyme substrate}$

$K_s, K_2, K_E, K'_s, K_4 = \text{equilibrium constant}$

$K_E, K'_s = K_{ES}, K_s$

$\frac{K'_s}{K_s} = \frac{K_{ES}}{K_E} = \frac{[EHS^*]}{[H^+][EH]}$

$K_E = \frac{[H^+][EH]}{[E][S^*]}, K_{ES} = \frac{[EHS^*]}{[ES^*]}$

Rate of product formation is given by,

$$r_p = k_2 ES^* - k_4 EP \tag{16}$$

As enzyme is preserved;

$$E = E_0 - ES^* \quad (17)$$

Substituting Equation 17 into Equation 16, we get,

$$\begin{aligned} r_p &= K_2 ES^* - k_4 P (E_0 - ES^*) \\ r_p &= k_2 ES^* - k_4 P E_0 + k_4 P ES^* \\ r_p &= ES^* (k_2 + k_4 P) - k_4 P E_0 \end{aligned} \quad (18)$$

When the reaction is in equilibrium;

$$ES^* = \frac{k_s}{k'_s} E S^* \quad (19)$$

As ES^* is an enzyme-substrate complex and since there is no method to measure the complex, substituting Equation 17 into equation 19 and rearranging;

$$ES^* = \frac{S^* E_0}{\left(\frac{k'_s}{k_s} + S^* \right)} \quad (20)$$

Substituting Equation 20 into Equation 18;

$$\begin{aligned} r_p &= (k_2 + k_4 P) \frac{S^* E_0}{\left(\frac{k'_s}{k_s} + S^* \right)} - k_4 P E_0 \left(\frac{k'_s}{k_s} + S^* \right) \\ r_p &= \frac{k_2 E_0 \left(S^* - \frac{k_4 k'_s}{k_2 k_s} P \right)}{\frac{k'_s}{k_s} + S^*} \end{aligned} \quad (21)$$

But the reaction for complex enzyme-substrate, ES^* is much faster than the reaction for product formation, $E + P$. Therefore k_s and k'_s are much greater than the values of k_2 and k_4 . Hence Equation 21 could be simplified as;

$$r_p = \frac{k_2 E_0 S^*}{\left(\frac{k'_s}{k_s} + S^* \right)} \quad (22)$$

In terms of rate of reaction;

$$r = r_p = \frac{dP}{dt} = -\frac{dS}{dt} = \frac{k_2 E_0 S^*}{\frac{k'_s}{k_s} + S^*} \quad (23)$$

If Equation 23 is simplified further with introducing a new parameter which is; $k_m = \frac{k'_s}{k_s} = \frac{[EHS^*]}{[H^+][EH]}$, related to bulk

substrate concentration $[S^*]$ which is glucose, where concentration of enzyme protonated complex $[EHS^*]$, and concentration of enzyme protonated $[EH]$ are much smaller relative to $[S^*]$. According to Shuler and Kargi (2000), k_m is solely a function of rate parameters and is expected to change with temperature or pH. For $V_m = k_2 E_0$ as a lumped parameter since it was difficult to express E_0 in a molar unit, related to amount of enzyme used i.e. immobilized glucose isomerase. An enzyme is expressed in activity rather than in molar unit, due to the fact that the exact molecular weight of enzyme and an amount of pure enzyme was unknown. The final rate of the reaction could be expressed as below;

$$r = \frac{dP}{dt} = -\frac{dS}{dt} = \frac{V_m S^*}{k_m + S^*} \quad (24)$$

Rearranging Equation 24 into Equation 13, a relationship for temperature and pH of bulk substrate with rate of reaction is as follows;

$$r = \frac{[v_0 e^{-\frac{E_a}{RT}}][e^{-k_d t} e^{-\frac{E}{RT}}] S^*}{\frac{[EHS^*]}{[H^+][EH]} + S^*} \quad (25)$$

Since concentration of enzyme protonated complex, $[EHS^*]$ and concentration of enzyme protonated, $[EH]$ are much smaller relative to $[S^*]$, we could ignore it, thus $k_m = \frac{k'_s}{k_s} = \frac{[EHS^*]}{[H^+][EH]}$

change to $k_m = \frac{1}{[H^+]}$, rearranging Equation 25;

$$r = \frac{[v_0 e^{-\frac{E_a}{RT}}][e^{-k_d t} e^{-\frac{E}{RT}}] S^* [H^+]}{k_m + S^* [H^+]} \quad (26)$$

Equation 26 is known as MM3 with a function of temperature, pH and bulk substrate concentration. Based on the mass balance for each type of reactors, a simple programming was done using MATLAB in order to simulate the model MM3 in the batch reactor. Figure 2.0 shows stages in the development of a mathematical model and simulation for glucose isomerisation process.

RESULTS AND DISCUSSION

Effect of temperature

The results of the modeling described are presented and discussed in the following sections. Temperature is one of the factors that influence the process whether it is

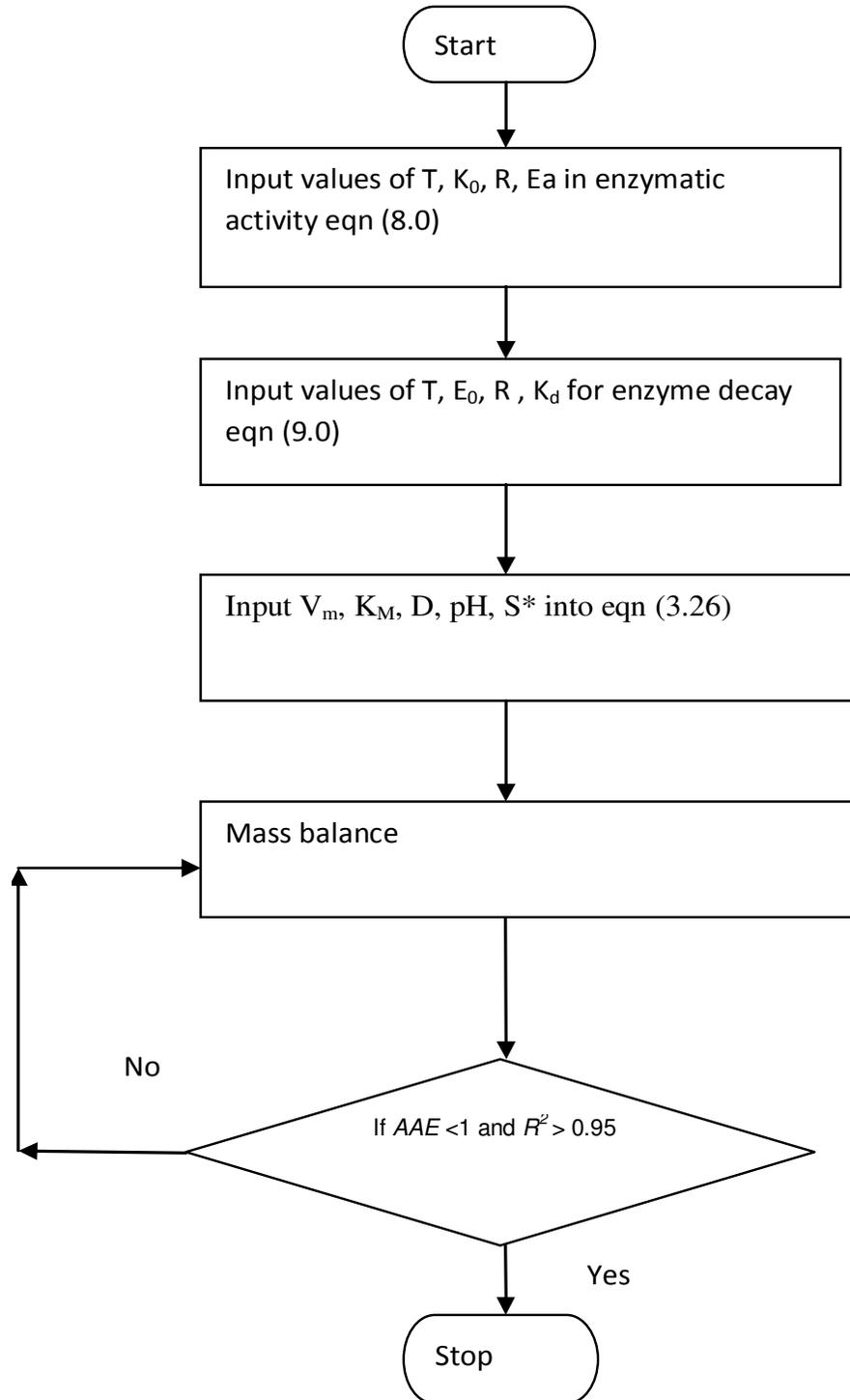


Figure 2. Flow chart for mathematical model and simulation of glucose isomerisation process.

batch or continuous. Equation 26 shows the temperature which affects enzyme activation and inactivation simultaneously, was the one of the main objectives in this study. In order to show the validation of the modeling and simulation, the following statistical analysis, such as correla-

tion of values, (R^2), average absolute error (AAE), Integral absolute error (IAE) and Integral square error (ISE) were used.

The kinetic parameters are initial rate of reaction, V_m , and Michaelis-Menten constant, K_M . The kinetic parameters

Table 1. Kinetic parameter at various temperatures.

Temperature (°C)	V _{max} (g/Lmin)	K _M (g/L)
55	1.823	1.041
60	1.859	1.107
65	1.929	1.239
70	0.374	0.353

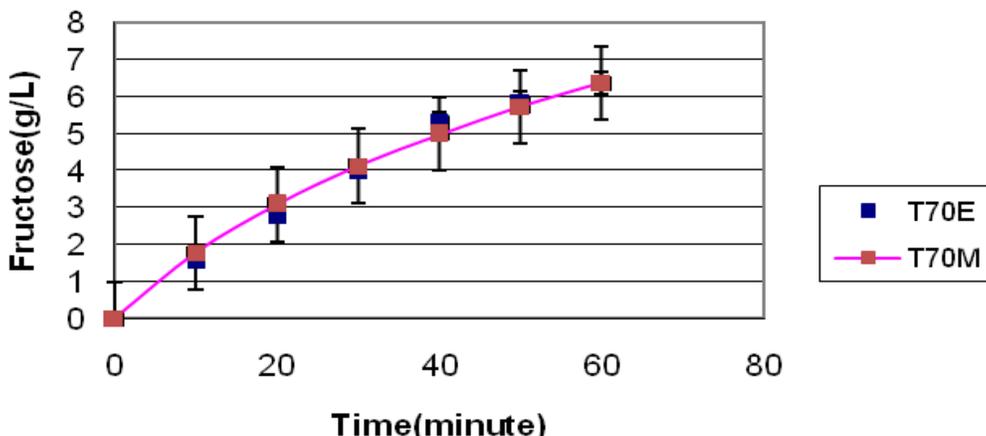


Figure 3. Effect of temperature (70°C) on the product formation, fructose (agitator speed, $\psi = 150$ rpm, pH=8).

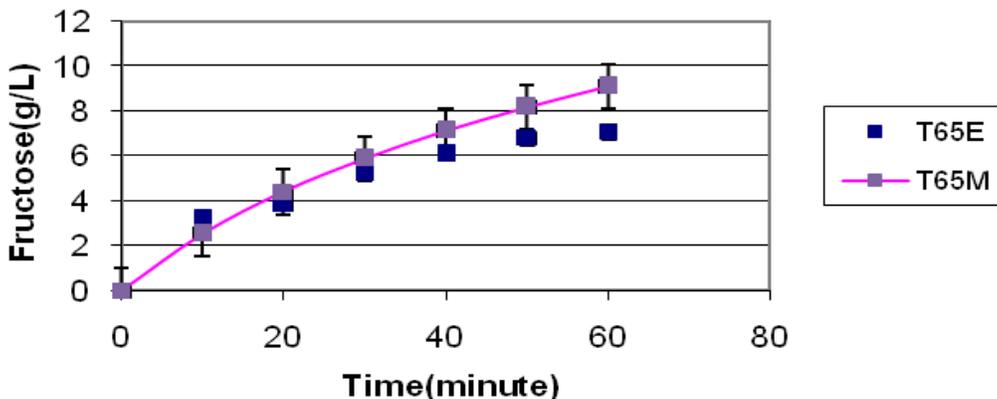


Figure 4. Effect of temperature (65°C) on the product formation, fructose (agitator speed, $\psi = 150$ rpm, pH=8).

are obtained using the Lineweaver plot and shown in Table 1. Perfect mixing between solid and liquid is the main assumption in this batch reactor. In order to get perfect mixing, the main characteristic of the mixer is to allow the solid to be just suspended in the liquid.

From the values of V_m and K_M for each temperature shown in Table 1, the simulation shows that the modified Santos-MM model (MM3) of Equation 26 proves that the activation and deactivation energy occurs simultaneously. The theory states that the degree of enzyme deactivation is four times greater than the activation energy (Shuler

and Kargi, 2002) and has been shown in the results. Figure 3 to 6 (with the error bars of 5 percent positive and negative potential error amounts) shows a comparison study of the effect of temperature between the proposed modified model (MM3) and the experimental results (E). Table 2 shows the correlation between modeling and the experimental as indicated by the correlation values (R^2), average absolute error (AAE), integral absolute error (IAE), and integral square error (ISE) for each temperature under study.

From Figures 3 - 6 and from Table 2, it shows that,

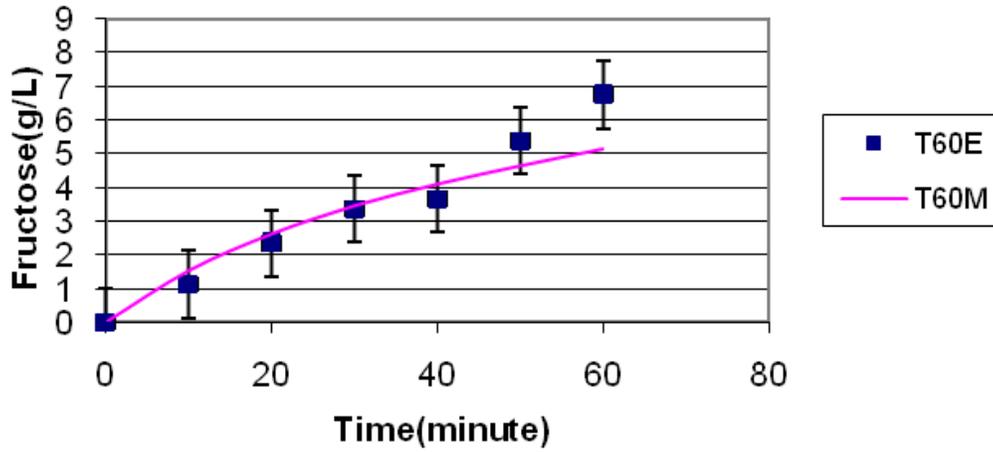


Figure 5. Effect of temperature (60°C) on the product formation, fructose (agitator speed, $\psi = 150\text{rpm}$, $\text{pH}=8$).

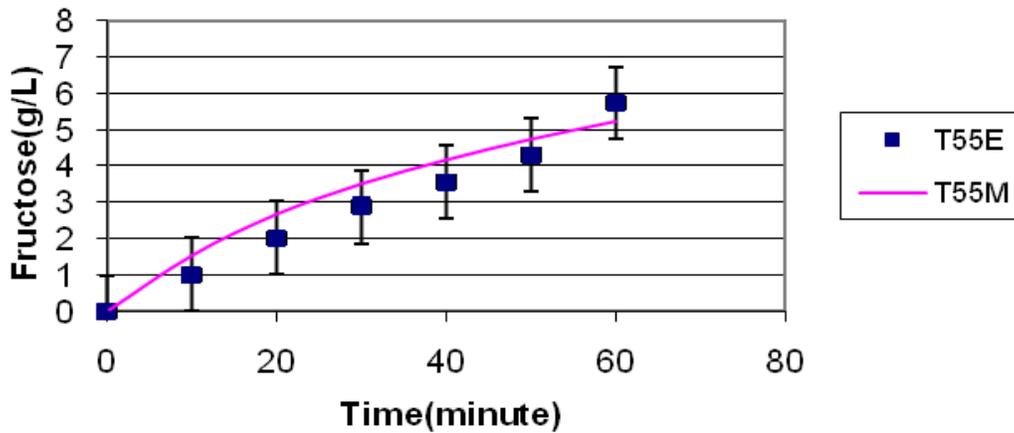


Figure 6. Effect of temperature (55°C) on the product formation, fructose (agitator speed, $\psi = 150\text{rpm}$, $\text{pH}=8$).

Table 2. The statistical analysis for temperature under study (Batch reactor).

Parameter	Temperature (°C)			
	55	60	65	70
R^2	0.974	0.964	0.984	0.997
Average absolute error (AAE)	0.123	0.046	0.148	0.009
Integral absolute error (IAE)	0.436	0.612	0.744	0.843
Integral square error (ISE)	0.668	0.856	0.940	0.978

for the temperature under study, there is a good correlation between the proposed model and the experimental results based on the range of R^2 from 0.964 to 0.997, The deviation of the proposed model, MM3 from experiment was measured with the error values which are AAE, IAE and ISE with the highest AAE at 0.148 and the lowest AAE at 0.009; IAE from 0.436 to 0.843 and ISE from 0.668 to 0.978. Even though the highest R^2 is at

70°C but based on the IAE of 0.843 and ISE of 0.978, it proved that for enzymatic reaction, it should be carried out below 65°C.

Another observation from these results is that, even though the initial rate of reaction, V_m , and Michaelis-Menten constant, K_M were maximum at 65°C through experimental works but for modeling, the highest correlation, R^2 occurred at 70°C not at 65°C. This could be explained

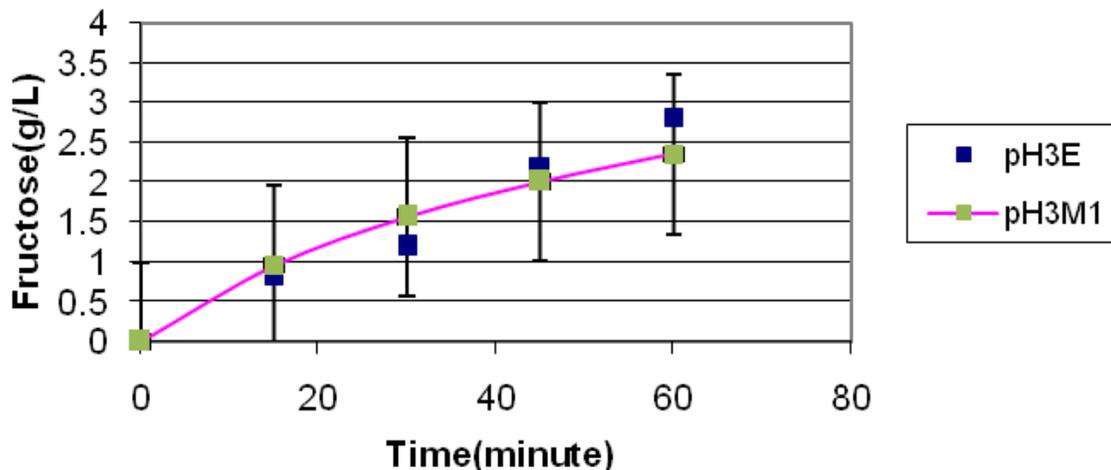


Figure 7. Effect of pH (3) on the product formation, fructose (agitator speed, $\psi = 150\text{rpm}$, 60°C).

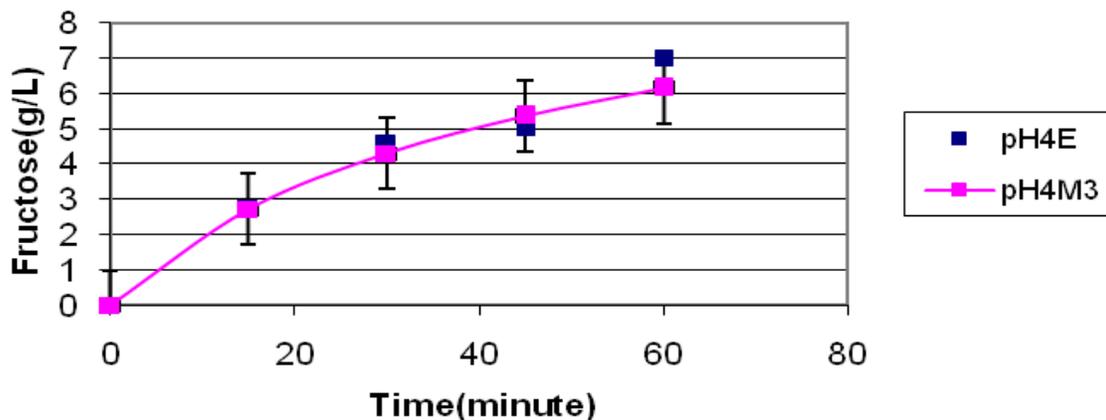


Figure 8. Effect of pH (4) on the product formation, fructose (agitator speed, $\psi = 150\text{rpm}$, 60°C).

in terms of diffusion of substrate to the active site of enzyme, increase in temperature would increase the diffusion of substrate as shown by Equation 3.22 (Fogler, 1999). Thus increase in temperature of substrate would increase the diffusivity of substrate and increase the product formation, regardless of enzyme decay. The effective diffusivity at 70°C was found to be at $1.166\text{ cm}^2\text{s}^{-1}$ whereas at 55°C , the value was $1.078\text{ cm}^2\text{s}^{-1}$.

Another reason for the difference between experimental and modeling could be explained in terms of IAE and ISE as shown in Table 2, which indicates IAE was 0.827 and ISE was 0.973 at 65°C . Hence it shows that the rate of reaction, r , which include V_m and K_M as in Equation 26 was more significant rather than considering only the initial rate of reaction V_m , as proposed by Santos (2007). The results also confirm that for enzymatic reaction, it should be carried out below 65°C since the IAE and ISE for 55 and 60°C gave the lowest values compared to others. These findings follow the theory which state that most proteins tend to decompose at temperature above 50°C (Baily and Ollis, 1986).

Effect of pH

The effect of acid H^+ ions or basic OH^- ions on the activity of an enzyme is probably caused by a change in configuration at or in the neighbourhood of the active sites. In modeling, the effect of pH in the process was mainly due to the values of kinetic parameters. The kinetic parameters are obtained using the Lineweaver plot. In this study, the pH range was from three to ten. According to Equation 26, the time τ , differ for each pH under study, which shows that the time taken for each acid H^+ ions or basic OH^- ions to move to the active site greatly influence the activity of an enzyme (Santos et al., 2007).

Figures 7, 8, 9, 10, 11, 12, 13 and 14 shows the results of the simulation for various pH's under study, where M in this figure refers to modeling and the term E refer to the experimental data. The trend of the pH profiles for modeling is quite similar to the experimental results as shown in those figures. The correlation and validation between modeling and the experiment is indicated by R^2 , AAE, IAE

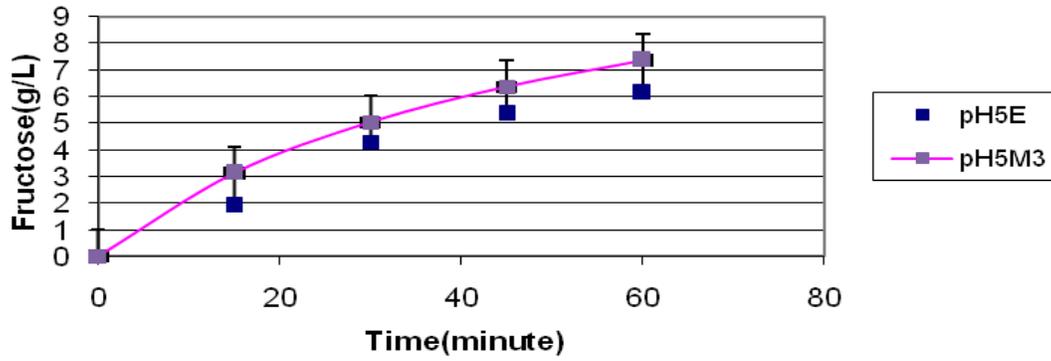


Figure 9. Effect of pH (5) on the product formation, fructose (agitator speed, $\psi = 150\text{rpm}$, 60°C).

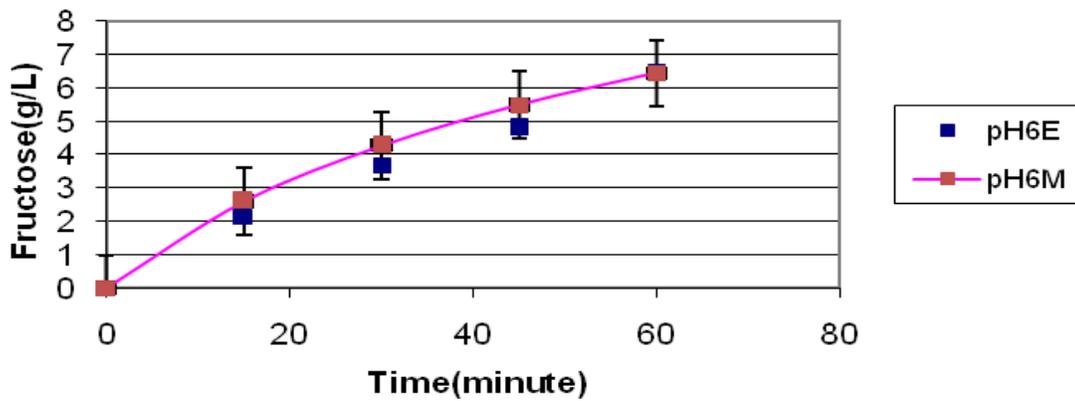


Figure 10. Effect of pH (6) on the product formation, fructose (agitator speed, $\psi = 150\text{rpm}$, 60°C).

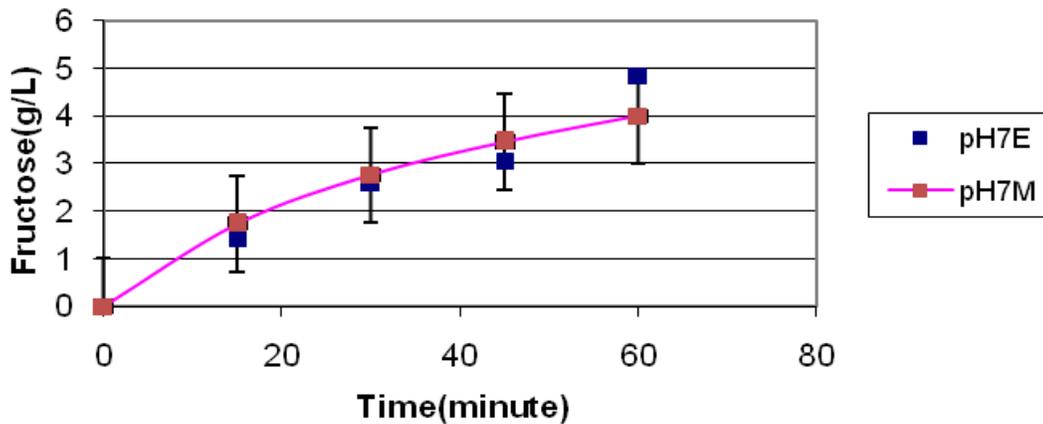


Figure 11. Effect of pH (7) on the product formation, fructose (agitator speed, $\psi = 150\text{rpm}$, 60°C).

and ISE for each pH under study, was shown in Table 3. From Table 3 and Figure 7 - 14, they show that, in general, there is a high correlation between the model and the experimental values. The results shows that the highest correlation shown by pH 9 (0.997) with the lowest by pH 8 (0.931). The highest IAE was at pH 4 (0.986) and the lowest was at pH 5 (0.359). For ISE, the lowest

values were at pH 5 (0.551) and for the highest it was at pH 4 (0.999).

Overall the AAE was less than one with the range from 0.003 to 0.237. Similar results are shown by the IAE and ISE and the R^2 was greater than 0.95, which indicates that the model MM3 was well fitted to the experiment data.

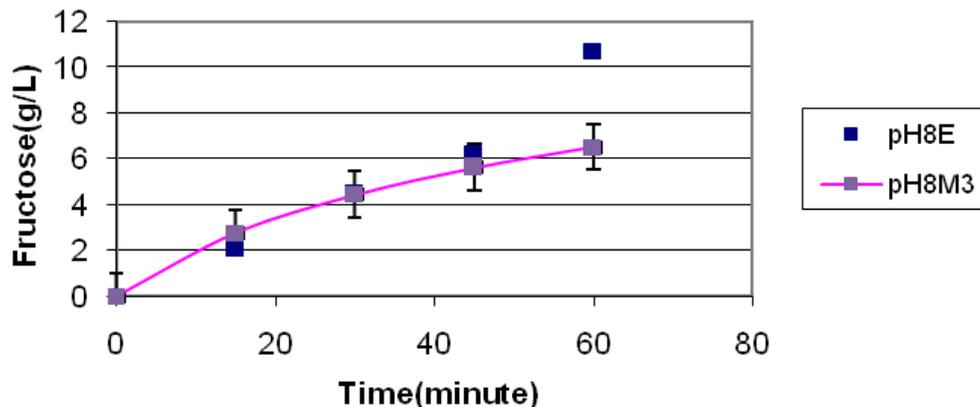


Figure 12. Effect of pH (8) on the product formation, fructose (agitator speed, $\psi = 150$ rpm, 60°C).

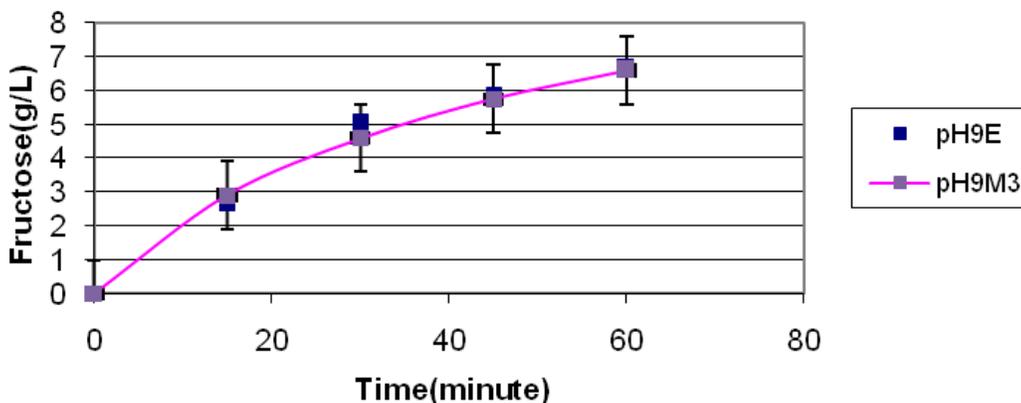


Figure 13. Effect of pH (9) on the product formation, fructose (agitator speed, $\psi = 150$ rpm, 60°C).

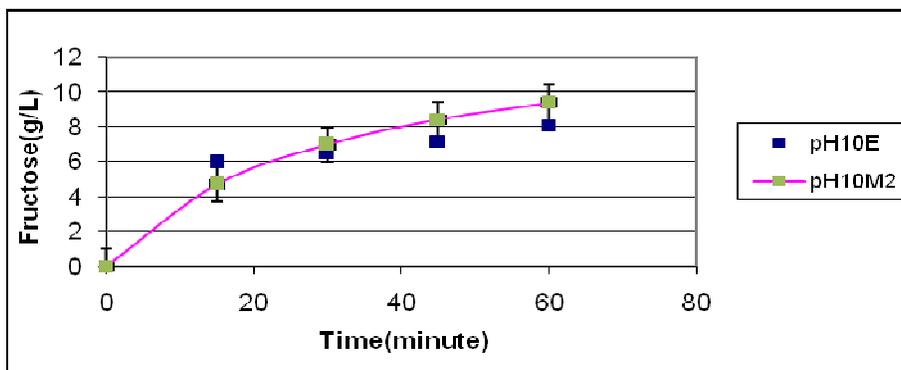


Figure 14: Effect of pH(10) on the product formation, fructose (agitator speed, $\psi = 150$ rpm, 60°C).

Validation with other model

The proposed model (MM3) was also compared with the Michelis-Menten (MM) model and Santos-Michelis-Menten model (SMM). The performance of each model was compared, based on the average absolute error

(AAE) and R^2 (Zajšek and Goršek, 2010). Table 4 shows that for each temperature, the values of fructose formation for MM3 was quite similar with the experimental results followed by SMM model and the greatest deviation was MM model. These proved that the MM3 was much better than the two models since all the condi-

Table 3. The statistical analysis for pH 3, 4, 5, 6, 7, 8, 9 and 10 (Batch reactor).

Parameter	pH							
	3	4	5	6	7	8	9	10
R ²	0.968	0.988	0.992	0.992	0.965	0.931	0.997	0.963
AAE	0.022	0.044	0.237	0.102	0.003	0.172	0.023	0.065
IAE	0.830	0.986	0.359	0.766	0.755	0.659	0.904	0.765
ISE	0.974	0.999	0.551	0.950	0.945	0.891	0.992	0.949

Table 4. A comparison study between various models on the average of fructose formation with the effect of temperature in batch reactor.

Temperature (°C)	Michelis-Menten model (MM)	Santos-Michelis-Menten model (SMM)	Proposed model (MM3)	Experiment (E)
55	0.721	7.003	3.130	2.877
60	0.704	6.251	3.084	3.231
65	1.686	11.061	7.971	4.628
70	0.78	3.723	3.723	3.69

Table 5. An analysts study between various models with the effect of temperature in batch reactor.

Parameter	Temperature (°C)	Michelis-Menten model (MM)	Santos-Michelis-Menten model (SMM)	Proposed model (MM3)
Average absolute error (AAE)	55	0.749	1.435	0.123
	60	0.782	0.935	0.046
	65	0.589	1.694	0.942
	70	0.789	0.009	0.196
R ²	55	0.959	0.971	0.974
	60	0.970	0.978	0.964
	65	0.918	0.918	0.984
	70	0.984	0.984	0.997

tions were similar for all models. This result was further analyzed in terms of average absolute error (AAE) and R² for each model, with the intention of showing that the MM3 is acceptable. From Table 5, it shows that AAE was highest for SMM model (1.694), followed by MM model (0.789) and eventually the lowest values (0.046) for MM3. The reasons for this results in SMM model, are mainly about K_M factor, since it doesn't engage the K_M factor for the overall rate of reaction (Santos et al., 2007). This results prove that the importance of V_m and K_M in developing the overall rate of reaction as shown by Equation 26 in MM3. All models show a very good correlation as the R² were above 0.9. As the R² were above 0.95 for all temperature in MM3, these demonstrate that MM3 was satisfactory.

It is always advisable to check the coherence between the experiment and a model, within its valid range, before the model can be considered "valid" (Faria et al., 2010).

By using the kinetic parameters in Table 1 for Michelis-Menten (MM) model, Santos-Michelis-Menten model (SMM) and the proposed model (MM3), a comparison for the validity of the model were conducted using Matlab software. Table 6 summarised the average of fructose formation for each model in the batch reactor.

From Table 6, it shows that for MM model, fructose formation were higher than the experimental work for all range of pH under study. These results proved that the activation energy and deactivation energy greatly influence fructose formation. For SMM model, the values obtained were much lower than the experimental works. Once again, this shows that the importance to include initial reaction rate and K_M for overall reaction rate which involved bulk substrate concentration which is not included in the SMM model. Comparing with the two models, the MM3 model was in a good agreement with the experimental works followed by SMM and MM. This is

Table 6. A comparison study between various model on the fructose formation with the effect of pH in a batch reactor.

pH	Michelis-Menten model (MM)	Santos-Michelis-Menten model (SMM)	Proposed model (MM3)	Experiment
3	14.239	0.342	1.377	1.408
4	14.252	0.999	3.707	3.876
5	14.394	1.132	4.375	3.537
6	13.326	0.094	3.759	3.412
7	14.268	0.392	2.385	2.378
8	14.366	1.006	3.874	4.681
9	14.316	0.896	3.964	4.055
10	14.159	1.818	5.894	5.534

Table 7. An analysts study between various model with the effect of pH for batch reactor.

Parameter	pH	Michelis-Menten model (MM)	Santos-Michelis-Menten model (SMM)	Proposed model (MM3)	
Average absolute error (AAE)	3	9.112	0.757	0.022	
	4	2.676	0.742	0.044	
	5	3.609	0.680	0.237	
	6	2.905	0.972	0.102	
	7	4.999	0.835	0.003	
	8	2.069	0.785	0.172	
	9	2.530	0.779	0.023	
	10	1.559	0.671	0.065	
	R ²	3	0.724	0.993	0.968
		4	0.837	0.997	0.988
5		0.779	0.980	0.992	
6		0.879	0.994	0.992	
7		0.749	0.987	0.965	
8		0.644	0.981	0.931	
9		0.843	0.969	0.997	
10		0.977	0.877	0.966	

shown by the analysis study as shown in Table 7. For average absolute error (AAE) and R² for each model, the results are presented in Table 7.

From Table 7, it shows that for average absolute error (AAE), in general, the range for MM model varies from 1.559 - 9.112, followed by SMM (0.671 - 0.972), and the MM3 model (0.003 - 0.237). For MM model the highest AAE was at pH3 (9.112) whereas for SMM model the value was 0.972 at pH 6 and for the proposed model the highest value was 0.237 at pH 5. This proves that the MM3 model was greatly superior compared to the other models. The R² results indicate a correlation between models and the experimental works. Therefore the results from Table 7 shows that in general, both SMM and MM3 models have a very good correlation since the values are above 0.95. As the R² for MM model was in a range 0.644 to 0.977 which was mostly below 0.95, this shows

that the MM model was inadequate.

Conclusion

The primary objective of this chapter is to compare and discuss the performance of all models (MM3, SMM and MM) in the batch reactor, employed in this simulation study, results and discussion were conducted based on the effect of temperature and pH to the models by varying kinetic parameter V_m and K_M , which were obtained through experimental works. The validation of the models with experimental data was checked in terms of AAE and R². Even though the highest R² was at 70°C, but based on the IAE of 0.843 and ISE of 0.978, it proved that for enzymatic reaction, it should be carried out below 65°C. Effect of pH for various models shown that, the IAE and

ISE were less than one whereas the R^2 were greater than 0.95. This indicates that the model MM3 is acceptable. All models show a very good correlation as the R^2 were above 0.9. As the R^2 were above 0.95 for all temperature in MM3, these demonstrate that MM3 was satisfactory.

ACKNOWLEDGMENT

The research is funded by Fundamental Research Grant Scheme (FRGS) by Ministry of Higher Education, MOHE (UKM-KK-02-FRGS0126-2009) which are duly acknowledged by authors.

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