Determination of Process Stability and Response for Glucose Isomerisation Process

Norliza Abd.Rahman (Corresponding author) & Jamaliah Md. Jahim
Department of Chemical Engineering & Process
Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia
43650 Bangi, Selangor, Malaysia
Tel: 60-3-8921-6413 E-mail: liza@eng.ukm.my

Mohd.Azlan.Hussain
Department of Chemical Engineering, Faculty of Engineering, Universiti Malaya
50603 Kuala Lumpur, Malaysia

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Abstract

Production of fructose from glucose isomerisation process using a commercial immobilized glucose isomerase (IGI), involved many factors such as pH, temperature, feed flow rate and initial glucose concentration. This study is focused on determination of process stability, eigenvalues,λ, and response of the process, eigenvector,ξ, at various space velocity, $D=F/V$ (F= feed flow rate mLmin^{-1} and V= volume of packed-bed reactor, 65mL) with initial steady state of glucose and fructose concentration in improving formation of fructose. Simplified Michelis-Menten model was derived for glucose isomerisation with initial concentrations of glucose from 18 gL^{-1}. The temperature under study was 60ºC with pH of 7 and D from 0, 0.3,1, 5 and 10 per minute. From the results, the steady- state of glucose concentration was obtained at 16.375 g/L and 1.75 gL^{-1}. At D = 0.3 the eigenvalues,λ, for glucose and fructose are–0.3 and –0.0002 which show that the process is stable as the eigenvalues is negative whereas the faster response is given by the eigenvector,ξ with values of [-0.3;0]. Increase D will increase the response of the process but at same time maintain the stability of the process.

Keywords: Glucose Isomerisation, Fructose, Immobilized glucose isomerase (IGI), Eigenvalues, Eigenvector

1. Introduction

Isomerisation of glucose to fructose by immobilized glucose isomerase enzyme is an example of a solid catalyzed bioreaction. Such reaction is industrially important and it is a reversible reaction with an equilibrium conversion of 50% at 65ºC [Salehi et al, 2004]. Comparable to the sucrose syrup, fructose has more desirable functional properties such as high osmotic pressure, high solubility, and a source of instant energy as well as preventing crystallization of sugar in food products [Kurup et al. , 2005]. An enzyme was used in this study as most of enzyme-based processes operate at lower temperatures, produce less toxic and pollutant waste; produce fewer emissions and by-products compared to conventional chemical processes [Bazaara et al.1996, Bhosale et al., 1996].

According to a glucose isomerase supplier, Novozymes , '*activity'* of the enzyme is defined, as the rate of conversion of glucose to fructose per mass of IGI, unit IGIU. The '*stability'* is the ability of the enzyme to retain its activity during extended operation. Both activity and stability are strongly affected by the operating temperature beside the formation of by-product. In this study, immobilized Glucose Isomerase (IGI) was from *S.murinus*, with activity of 350 IGIU/g (Sweetzyme, Novozymes). Bazaraa & Hassan (1996) in their study found that the optimum conditions for glucose isomerisation process were at 56^{o}C to 60^{o}C, pH of 7.4 to 7.8 and 34 to 36%w/w glucose content but the process under study was a continuous system. Prabhakar & Raju (1995) using a batch system with the glucose isomerase obtained from *Arthrobacter sp* that is different from the present study. No researchers mention above have shown whether the process is stable and which of the input variables would give a faster response. Bequette. (1998), Stephanopoulos. (1984) and Seborg et al. (2004) state that, stability of

the process is given by eigenvalues, λ, of a 'state variable' in a '**n x n**' matrix. The stable process is determined by the range of λ less than zero (<0). The response of the process can be shown by eigenvector, ξ. The magnitude of eigenvector would measure the response of the process. The faster response is given by the higher magnitude of eigenvector.

2. Methods

The organization of the paper is as follow. The first section involves experimental works carried out in a stirred tank and a packed-bed reactor with varied feed flow rate. The objective of experimental works carried out in a stirred tank was to determine the optimum initial substrate concentration. Using the result obtained from stirred tank, the experiment was conducted using packed-bed reactor in order to investigate the effect of various feed flow rate to the process. The following section is the calculation of stability and response of the process.

2.1 Materials

The materials for these studies are D-glucose (G), D+ fructose (F) and $MgSO_4.7H_2O$. They were obtained from R&M Chemical, UK. An amount of 12g IGI was used for stirred tank and 6g for packed-bed reactor. Different concentrations of glucose from 10 to 50% w/v were prepared. All analytical samples were diluted with distilled water and filtered through 0.45μm nylon filters prior to HPLC-analysis. Deionised water and acetonitrile (HPLC grade) were the mobile phase used for sample analysis using HPLC method.

2.2 Methods

(a) The reactor system

As shown in Figure 1, the reactor system consists of a 2 liter double –jacketed reactor, made from borosilicate glass 3.3 DN 120 043943. The reactor was connected to a water bath (Huber) and has a 3 bladed propeller type impeller driven by a motor (Heildoph with RZR323 control). The function of the water bath was to control the jacketed bioreactor at the required temperature. The feed consisted of 1 liter of solution containing 18g glucose and 1g of $MgSO_4.7H_2O$ (i.e.0.1M glucose and $1gL^{-1}$ $MgSO_4.7H_2O$ in distilled water (DW)). All the experiments were conducted at constant agitation speed of 150 rpm. The enzyme was dehydrated with distilled water for 24 hours in a cold room at 4ºC before being added to the reactor.

The packed-bed reactor is shown in Figure 2. The amount of IGI used in this reactor is 6g. 12g of IGI cannot be used in this type of reactor due to channeling flow of the substrate as a formation of compacted IGI. Consequently, imperfect distribution flow of substrate with enzyme. 9.4g of mesh wire was used as a bead. The feed flow rate was varied from 3 $mLmin^{-1}$ to 23.5 $mLmin^{-1}$. Optimum condition at 65°C and pH 8 based on the batch reactor system was used for operating this type of reactor. A certain amount of NaOH or HCl was added to the feedstock in order to reach the desired valued of pH. The temperature for the reaction was controlled using water bath connected to the jacketed reactor as shown in Figure 2.

The final rate of the reaction could be express as below;

$$r = \frac{dP}{dt} = -\frac{dS}{dt} = \frac{v_{\max} S}{k_m + S} \tag{1}$$

Equation (1) follows the Michaelis -Menten Kinetics. However Lee et al. (1979) shown that the Michaelis -Menten model is not 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 expressed as;

$$r = \frac{v_{\max}^{'} S^*}{k_m^{'} + S^*} \tag{2}$$

Where S^* is a bulk substrate concentration readily measurable compare to substrate concentration inside the catalyst and $v_{\max}^{'}$ and $k_m^{'}$ are the apparent kinetic constants. Enzyme deactivation in this model similar with the terms used by Asif & Abaseed [1998] and Santos et al. [2007] except that a relationship among overall reaction rate, temperature and decay constant k_d were introduced in this study. Enzymatic activity can be expressed by Equation (3) and K, the kinetic constant varies with temperature, therefore it can be expressed by an Arrhenius type equation (4).

$$v = KE \tag{3}$$

$$K = K_0 e^{-\frac{Ea}{RT}} \tag{4}$$

K is significant at the lowest temperature which indicate that it is the activation process.

For enzyme decay rate; $\frac{dE}{dt} = -k_d E_a$ (5)

From equation (5), rearranging;

$$E = E_0 e^{-k_d t} \tag{6}$$

Where k_d is a denaturizing constant and temperature dependent and E is significant at the highest temperatures that is the inactivation process. Applying Arrhenius type equation to k_d ,

$$K_d = K_{d0} e^{-\frac{E_d}{RT}} \tag{7}$$

Substituting equation (4), (6) and (7) into (3) gives the final expression for v as a function of temperature and reaction time.

$$v = [K_0 e^{-\frac{E_a}{RT}}][E_0 e^{-(k_{d0} e^{-\frac{E_d}{RT}})t}] \tag{8}$$

t in equation (8) should be very small, less or equal to one (≤ 1) min since only initial rates are being considered. Since K_0 and E_0 are constants, they were lumped into a constant nominated, v_0, Equation (8) change to Equation (9).

$$v = [v_0 e^{-\frac{E_a}{RT}}][e^{-(k_{d0} e^{-\frac{E_d}{RT}})t}] \tag{9}$$

Nevertheless, v is actually present in the general form of reversible Michaelis-Menten kinetics, as in Equation (2). Substitute Equation (9) into Equation (2) leads to Equation (10) which represent the rate of reaction of the process which involved the bulk substrate concentration, S^*, $v'_{\max}$ and k'_m which are the apparent kinetic constants.

$$r = \frac{[v_0 e^{-\frac{E_a}{RT}}][e^{-[k_{d0} e^{-\frac{E}{RT}}]t}]S^*}{k'_m + S^*} \tag{10}$$

Equation (10) represents the rate of reaction as a function of higher temperature, lower temperature and bulk substrate concentration.

In order to determine the transfer function of the process, material balance for substrate (glucose) and product (fructose) was calculated as following;

Fructose material balance; (Fructose=*P*)

Rate of accumulation= in by flow – out by flow + generation

$$V\frac{dP}{dt} = FP_f - FP + Vr_1$$

Glucose material balance ;(glucose=S)

$$V\frac{dS}{dt} = FS_f - FS - Vr_2$$

Assuming a constant volume, $D=F/V$ and $r_1=r_2=r$

$$\frac{dP}{dt} = DP_f - DP + r_1$$

$$\frac{dS}{dt} = DS_f - DS - r_2$$

If P_f= 0, since no fructose in the feed

$$\frac{dP}{dt} = -DP + \frac{[v_0 e^{-\frac{Ea}{RT}}][e^{-[kd0\, e^{-\frac{Ed}{RT}}]t}]S}{k_m' + S}$$

For steady-state solution, the conditions are;

$$\frac{dP}{dt} = 0$$

$$\frac{dS}{dt} = 0$$

The stability of the non-linear equations can be determined by finding the following state-space form;
X'= Ax+Bu
Where X'= the derivative with respect to time
x=state variables,u= input variables
A, B= Jacobian matrix
If eigenvalues, λ of A is negative, it shows that the point is stable. The eigenvector ξ will determine the speed of the response.
For control purpose, the state –space was converted into transfer function

$$\dot{X} = Ax + Bu$$

Y = CX
Where X= the derivative with respect to time

x = state variables = $\begin{bmatrix} P - P_s \\ S - S_s \end{bmatrix}$

P_s and S_s are the variables at steady-state.

u= input variables = $\begin{bmatrix} D - D_s \\ S - S_s \end{bmatrix}$

C = [1 0; 0 1], A= [*-D V'; 0 –V'*], B= [*-P V'; Ss – S -D-V'*].

Ds and *Ss* are the variables at steady state. Using Matlab software, the transfer functions were obtained as follow;

For fructose; $g11(s) = -1.7s/s^2 + 0.3s + 0.0001$ (11)

For glucose; $g21(s) = 1.625s + 0.4875 / s^2 + 0.3s + 0.0001$ (12)

The poles of the characteristic polynomial;

$$s^2 + 0.3s + 0.0001 \quad (13)$$

Using the software Matlab/Simulink, the effect of various *D* to the stability and response of the process was determined using eq (11), (12) and (13).

3. Results and Discussion

From Figure 3 it can be seen that increase in the glucose concentration would increase the formation of the fructose gradually. The results obtained follow Michaelis-Menten equation (2), where concentration of substrate is directly proportional to the rate of reaction. The substrate concentration of 30% w/v produce fructose at 0.95 $molL^{-1}$ compare to 1.05 $molL^{-1}$ if the substrate concentration was conducted at 50% w/v and at 0.65 $molL^{-1}$ if it was at 40% w/v. This shows that using the initial substrate concentration of 30% w/v was sufficient for the process. Since this is an enzymatic reaction, an important feature to be identified was the activity of enzyme. In this study, the activity of IGI was known as IGIU. From initial reaction rate that is the slope of the graphs above, the activity at various glucose concentrations was calculated by taking into account the initial quantity of glucose (20g) and IGI used (2g) as shown in Table 1.

From Table 1, it shows that the activity of IGI increase with increase of initial glucose concentration except for the 20% w/v of initial glucose concentration. These can be explained from the lock and key hypothesis for an enzymatic reaction. The results occur because the substrate was bonded by a weak attractive forces or covalent bond, to a specific region called active site of an enzyme where the reaction occurs and products are released.

The capacity for the products formations depends on the active site of the enzyme. If the initial substrate concentration was too high, it would inundate an enzyme and hence blocked the active site of an enzyme which shown in Figure 4.

Table 2 shows the result of stability and response for the simplified Michaelis-Menten model within a range of *D*. Increasing *D*, both substrate eigenvalue λs, and product eigenvalue λp, still preserve the stability of the process. The substrate eigenvalue λs increasing akin with the values of *D*. Product stability, λp, remain constant at -0.0002 after the values of *D* vary from 0 to 0.3. As *D* is a function of feed flow rate and volume of reactor, changing *D* means altering the feed flow rate, as the volume of reactor is kept constant. Therefore, the stability of substrate follows the values of *D*.

Response of the process is given by the eigenvector, ξ. The magnitude of eigenvector would measure the response of the process. The faster response is known by the higher magnitude of eigenvector. The faster response arise when the value of *D* is at 10, which shows that increasing the feed flow rate, tends to accelerate response of the process but at the same time uphold the stability of the process. Figure 5 shows that with faster response the fructose produced also increased. At $D = 10$, fructose formation was at 18 gL^{-1} whereas at $D = 5$, only 7 gL^{-1} of fructose have been obtained. With initial estimate of substrate concentration, $Cs = 18$ and product concentration, $C_p = 0$, gave the result for steady state as shown in Figure 5 with $C_s = 16.2463$ and Cp = 1.7537.

4. Conclusion

From the results presented above it can be concluded that by increasing *D* with time would increase the response of the process but at the same time preserve the stability of the process. Increase the substrate concentrations; increase the rate of reaction and enzyme activity. The optimum value of initial substrate concentration was at 30% w/v to produce fructose at 0.95 $molL^{-1}$. The faster response occurs when the value of *D* is at 10 and product stability, λp, remain constant at -0.0002 after the values of *D* vary from 0 to 0.3.

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Table 1. Effect of initial glucose concentration to the initial rate and enzyme activity

Initial Glucose concentration			Slope	Initial Rate	Activity
Test no	% w/v	mol/l	mol/l/min	umol/min/l	IGIU/G
1	10	0.56	4.90E-03	4900	123
2	20	1.11	8.60E-03	8600	215
3	30	1.67	0.011	11000	275
4	40	2.22	0.0123	12300	308
5	50	2.78	0.0137	13700	343

Table 2. Stability and response using the Cs= 16.2463 and Cp = 1.7537

F/V	Stability,λ	Response,ξ
0	λs =0, λp=-0.2374	ξs = [0 0], ξp=[0.2374 0.2374]
0.3	λs =-0.3, λp=-0.0002	ξs = [-0.3 0], ξp=[0.0002 0.0002]
1	λs =-1, λp=-0.0002	ξs = [-1 0], ξp=[0.0002 -0.0002]
10	λs =-10, λp=-0.0002	ξs = [-10 0], ξp=[0.0002 -0.0002]

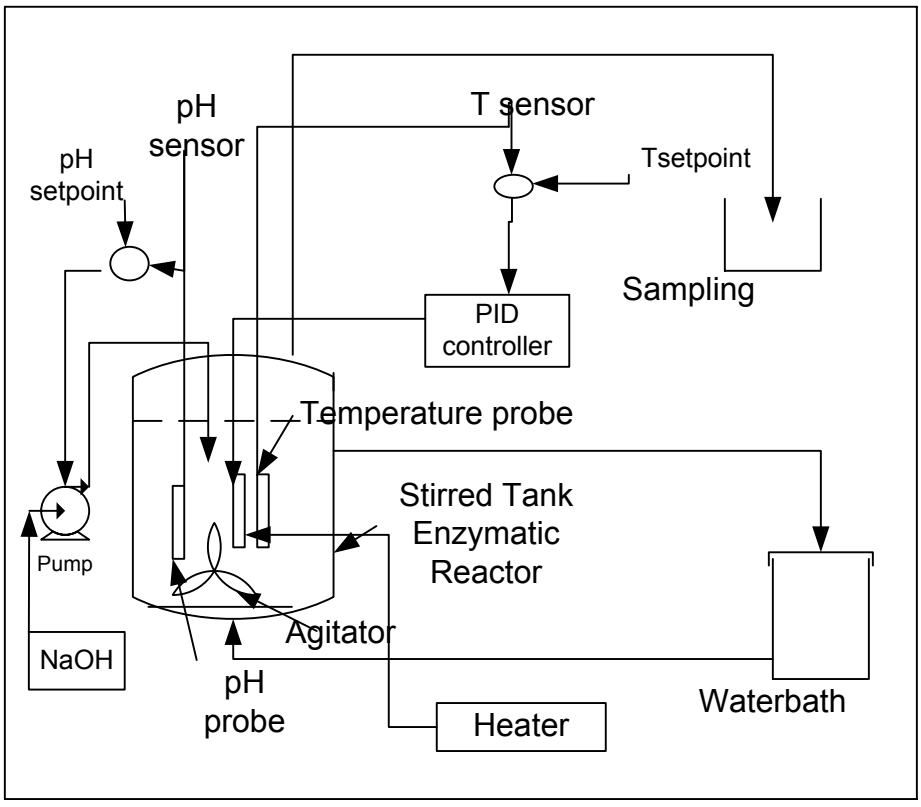

Figure 1. Schematic diagram for Stirred-tank glucose isomerisation process

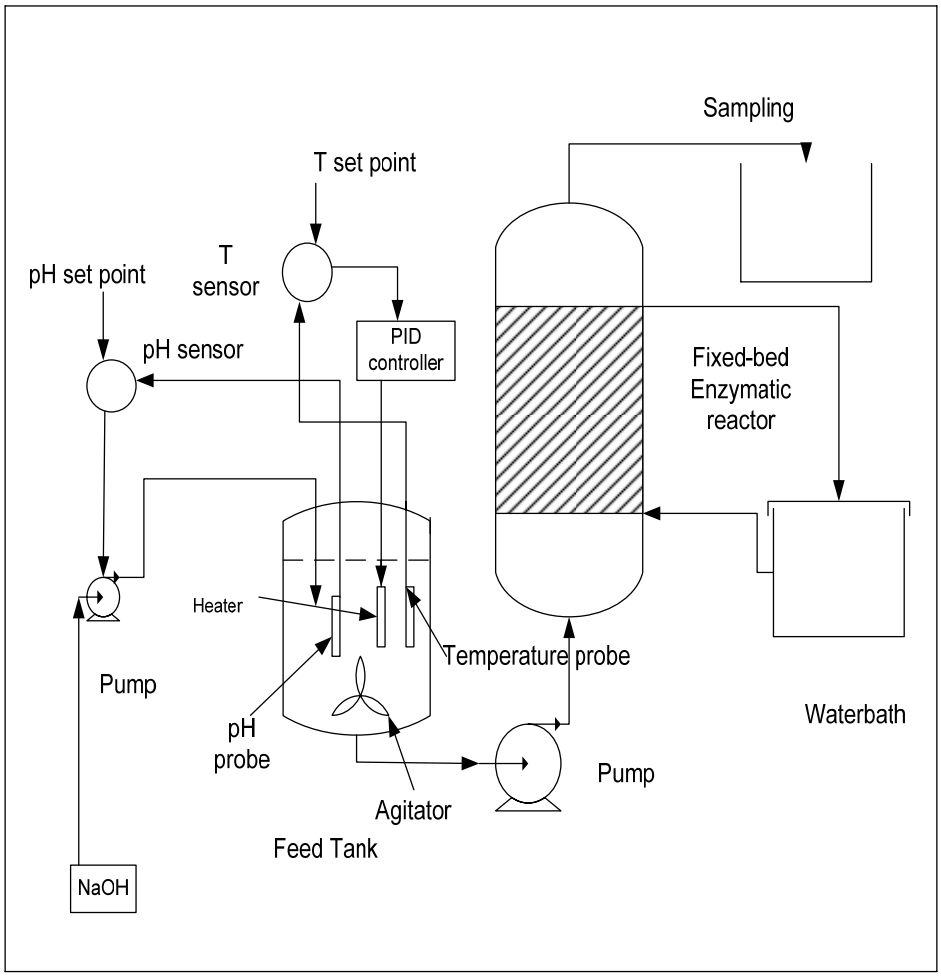

Figure 2. The schematic diagram for glucose isomerisation process with packed-bed reactor

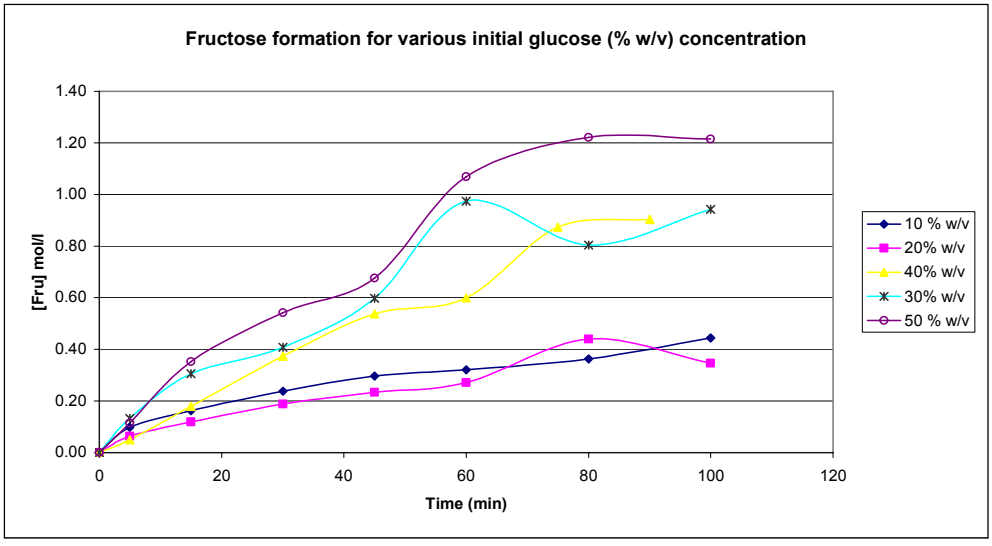

Figure 3. Fructose formation for various initial glucose concentrations (%w/v)

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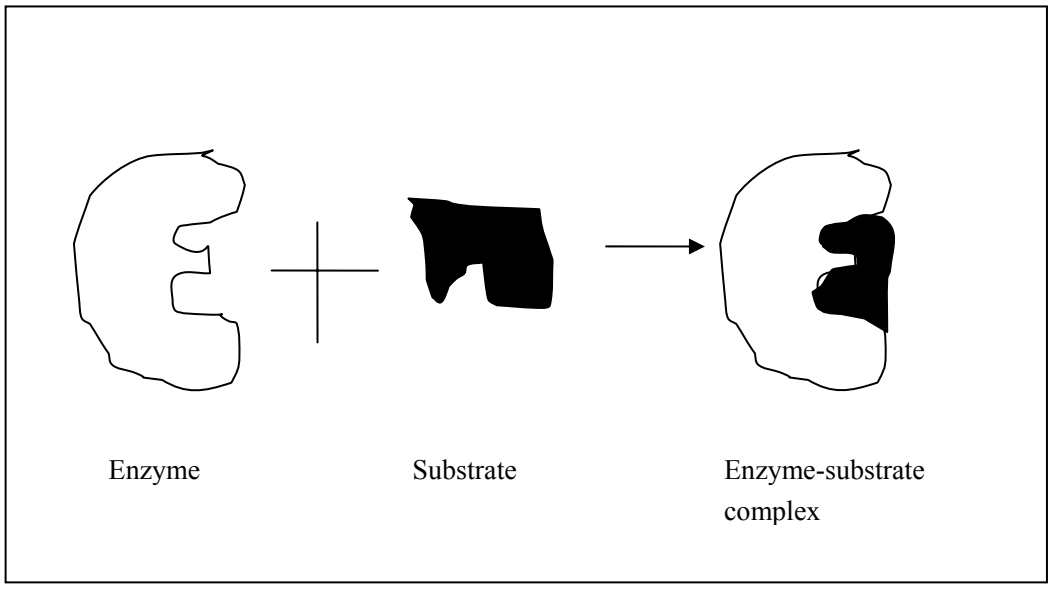

Figure 4. Lock and key theory for the enzyme- substrate complex (Lee, 1992)

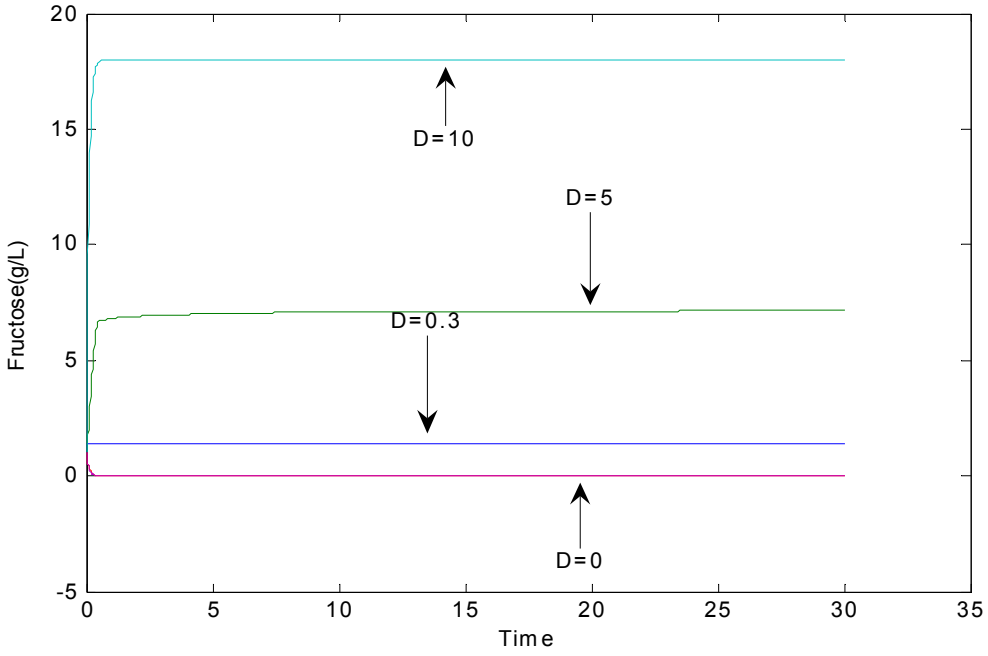

Figure 5. Fructose formation for several of D