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ARTICLE *in* INDUSTRIAL & ENGINEERING CHEMISTRY RESEARCH · MARCH 2009

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Ind. Eng. Chem. Res., **Article ASAP** • DOI: 10.1021/ie800400b • Publication Date (Web): 11 February 2009

Kinetics of Glucose Isomerization to Fructose by Immobilized Glucose Isomerase (Sweetzyme IT)

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We present the kinetic parameters and equilibrium constant of the enzymatic glucose−fructose isomerization reaction with an immobilized glucose isomerase (IGI), Sweetzyme IT, using a batch stirred-tank reactor following the procedure developed by Dehkordi et al. (*AIChE J.* 2008, *54*, 1333). The model predictions were compared with the experimental data and fair agreements were found. The influence of temperature on the equilibrium constant and kinetic parameters of glucose to fructose isomerization reaction was investigated. In addition, the frequency factors and the activation energies were determined by using Arrhenius-like expressions. Furthermore, correlations were found for the maximum reaction rate (i.e., V_m) and apparent Michaelis−Menten constant (i.e., K_m).

Introduction

The isomerization of glucose to fructose is one of the most widely used processes in the food industry in producing dietetic "light" foods and drinks because it improves the sweetening, color, and hygroscopic characteristics in addition to reducing viscosity. Moreover, fructose is about 75% sweeter than sucrose, is absorbed more slowly than glucose, and is metabolized without the intervention of insulin. For all these reasons, this process is widely studied both with cells and with enzymes, both free and immobilized.[2−17]

In our pervious work,[1] it was shown that there are slight deviations from the Michaelis−Menten kinetic model at low fractional conversions (i.e., <10%) and hence a new procedure was developed to evaluate the kinetic parameters of glucose to fructose isomerization reaction at 60 °C satisfactorily. Following our previous work, an investigation was conducted on the enzymatic isomerization of glucose to fructose using a commercial immobilized glucose isomerase catalyst, Sweetzyme IT, over the reaction temperature range of 50−65 °C and glucose concentration range of 0.1−1.25 M. Moreover, to investigate the kinetics of immobilized enzymes, it is essential to have a reactor system in which there is no external mass-transfer resistance to the catalyst particles. It was thus decided to use a batch stirred-tank reactor.

Experimental Section

Chemicals. All chemicals used in the present study were of analytical grade; D-glucose in crystalline form was provided by Merck Co. (Germany). The immobilized enzyme, Sweetzyme IT, was provided as a gift by Novo Nordisk (Iran). The immobilized glucose isomerase (IGI) enzyme particles were of cylindrical shape, with 0.2−0.4 mm diameter, 1−1.5 mm length, and a particle density of 3300 kg m^{-3}. The dry specific activity of the IGI enzyme was reported to be 450 IGIU/g by the manufacturer. The microorganism of the IGI enzyme used in the present work is a selected strain of *Streptomyces murinus*. Moreover, the distilled water used was with conductivity ≤3 μS/cm.

Method of Analysis. Fructose and glucose concentrations were determined by HPLC (Waters, refractive index detector 2410). The Sugar Pak I column was used with deionized water as the mobile phase at a flow rate of 0.34 mL min^{-1}. The HPLC detector was calibrated by introducing known samples of D-glucose and D-fructose solutions. The regression coefficient of the calibration curve of the detector was 0.996. All glucose and fructose analyses were repeated in duplicate.

Experimental Apparatus. The batch reactor was a 500 mL jacketed stirred-tank reactor. The reactor temperature was adjusted by means of hot water. The heating system was able to adjust the temperature of the reactor with the accuracy of ±1 °C. Two connections located on the top of the reactor were provided (1) to introduce the desired amount of fresh catalyst to the reactor at the start of each experimental run; and (2) to withdraw samples from the reactor. The impeller was of flat-blade turbine type made of stainless steel and its rotation speed was adjusted from 100 to 1000 rpm by a variable-speed electric motor.

Experimental Procedure. The experimental runs were carried out at various temperatures, ranging from 50 to 65 °C. For each temperature, different initial concentrations of glucose ranging from 0.1 to 1.25 M were tested whereas the catalyst loading was varied over the range of 5−42 g/L. All the experiments were carried out at a constant pH of 7.5 and the duration of 120 min.

The D-glucose solution was prepared by dissolving the required amount of D-glucose in a solution containing 2.465 g

Table 1. Kinetic Constants of Eq 8

$[G]_0$ (mol/m^3)	T (°C)	K	$K_r \times 10^6$ (mol/(g_{cat}·s))
100	50	−0.386	3.869
500	50	−0.546	6.461
750	50	−0.579	6.520
1250	50	−0.750	7.575
100	55	−0.210	4.354
500	55	−0.382	8.276
750	55	−0.404	8.254
1250	55	−0.588	10.045
100	60	−0.073	2.508
500	60	−0.202	6.654
750	60	−0.252	11.683
1250	60	−0.300	11.032
100	65	−0.042	2.178
500	65	−0.127	9.681
750	65	−0.163	17.390
1250	65	−0.205	18.464

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10.1021/ie800400b CCC: $40.75

Table 2. Equilibrium Conversion, Equilibrium Constant, and Kinetic Parameters of Eqs 4 and 5

T (°C)	X_e	K_e	k_{mg} (mol/m^3)	k_{mf} (mol/m^3)	$\upsilon_{mg} \times 10^6$ (mol/(g_{cat}·s))	$\upsilon_{mf} \times 10^6$ (mol/(g_{cat}·s))	k_{mg}/k_{mf}	$\upsilon_{mg}/\upsilon_{mf}$
50	0.42	0.72	80.382	251.664	2.060	8.903	0.319	0.231
55	0.46	0.85	151.380	330.980	4.833	12.404	0.457	0.390
60	0.50	1.00	497.260	822.804	8.104	13.409	0.604	0.604
65	0.54	1.17	671.770	944.786	16.925	20.277	0.711	0.835

Table 3. Activation Energies, Frequency Factors of Kinetic Parameters, and Equilibrium Constant

parameter	value
E_{kmg}(J/gmol)	137380
E_{kmf} (J/gmol)	124150
E_{vmg}(J/gmol)	99000
E_{vmf}(J/gmol)	46190
E_{Ke} (J/gmol)	29220
k_{mg0} (mol/L)	1.32×10^{24}
k_{mf0} (mol/L)	5.20×10^{16}
υ_{mg0} (mol/(g_{cat}·s))	2.58×10^{14}
υ_{mf0} (mol/(g_{cat}·s))	2.64×10^{2}
K_{e0}	2.20×10^{4}

of $MgSO_4 \cdot 7H_2O$ per liter of deionized water to stabilize the enzyme; the pH of the solution was adjusted at 7.5 by Na_2CO_3. Because oxygen in the syrup inactivates the enzyme and is responsible for increased formation of secondary products during isomerization, a low oxygen tension thus has to be achieved by adding Na_2SO_3. Note that sodium sulfite is a highly effective oxygen scavenger and reacts chemically with dissolved oxygen, producing sodium sulfate.

In each experimental run, the feed solution with desired volume, concentration, temperature, and pH was fed to the reactor. Afterward, the impeller speed was adjusted at 700 rpm and the temperature of the reactor was kept at the desired temperature. Then the desired amount of IGI catalyst was suddenly added to the reactor. This time was considered as the starting time of the reaction. During the course of the reaction, samples were taken through the sampling connection by means of a syringe equipped with a filter to separate the catalyst. The progress of the reaction within the sampling bottles was ceased by adding sulfuric acid solution. Analysis of the samples was performed by the aforementioned analytical method for the glucose−fructose concentrations.

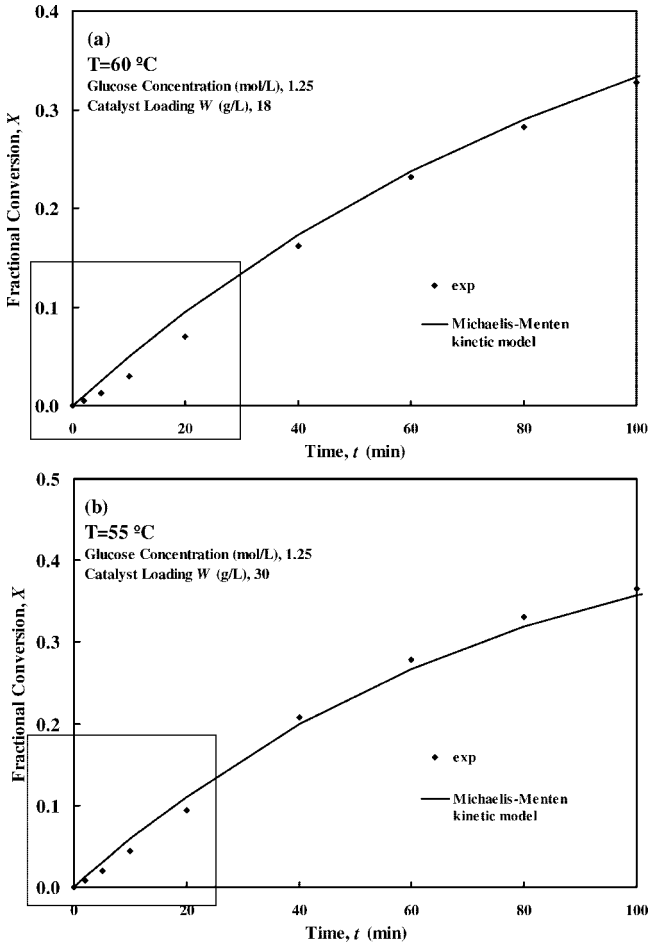

Figure 1. Variations of fractional conversion of glucose with time.

For each data point, the experimental run was repeated at least two times, and thus each data point was determined based on the mean value of at least two measurements of glucose−fructose concentrations with a standard deviation of 1−2%.

Reaction Kinetics. Glucose−fructose enzymatic isomerization is a reversible reaction and is normally given by the following expression:

$$\mathrm{G} + \mathrm{En} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{GEn} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{F} + \mathrm{En} \tag{1}$$

where G, En, and F represent glucose, enzyme, and fructose, respectively, and GEn is an intermediate complex formed during the reaction. According to the reversible modified Michaelis−Menten mechanism,[2,12,18,19] the reaction rate is generally given by

$$r_G = -\frac{1}{W}\frac{d[\bar{G}]}{dt} = \frac{V_m[\bar{G}]}{K_m + [\bar{G}]} \tag{2}$$

with

$$[\bar{G}] = [G] - [G]_e, [G]_0 = [G] + [F] = [G]_e + [F]_e = (1 + K_e)[G]_e = (1 + K_e^{-1})[F]_e \tag{3}$$

$$K_m = \frac{k_{mf}k_{mg}}{k_{mf} - k_{mg}}\left[1 + \left(\frac{1}{k_{mg}} + \frac{K_e}{k_{mf}}\right)[G]_e\right] \tag{4}$$

$$V_m = [1 + K_e^{-1}]\frac{k_{mf}\upsilon_{mg}}{k_{mf} - k_{mg}} \tag{5}$$

$$K_e = \frac{[F]_e}{[G]_e} = \frac{X_e}{1 - X_e} = \frac{\upsilon_{mg}k_{mf}}{\upsilon_{mf}k_{mg}} \tag{6}$$

where υ_{mg}, υ_{mf}, k_{mg}, and k_{mf} are the maximum reaction rate for glucose to fructose, the maximum reaction rate for fructose to glucose, the Michaelis−Menten constant for glucose to fructose reaction, and the Michaelis−Menten constant for fructose to glucose reaction, respectively, and X_e is the equilibrium fractional conversion of glucose.

It should also be added that V_m is the maximum reaction rate and K_m is the Michaelis constant. The reaction rate increases with increasing glucose concentration [G], asymptotically approaching the maximum rate, i.e., V_m. Moreover, a more appropriate measure to characterize an enzyme is the glucose concentration at which the reaction rate reaches half of its maximum value (i.e., $V_m/2$). This concentration can be shown to be equal to the Michaelis constant (i.e., K_m). On the other hand, the isomerization of glucose to fructose is a reversible reaction and hence we have two kinetic parameters for glucose

to fructose reaction (i.e., forward reaction), which are termed υ_{mg} and k_{mg}, and corresponding kinetic parameters for the reverse reaction (i.e., fructose to glucose reaction). The kinetic parameters of the reverse reaction are termed υ_{mf} and k_{mf}.

Integrating eq 2 gives

$$t = \frac{1}{W}\left[\frac{[\bar{G}]_0 - [\bar{G}]}{V_m} + \frac{K_m}{V_m}\ln\frac{[\bar{G}]_0}{[\bar{G}]}\right] \tag{7}$$

Thus, by introducing the values of υ_{mg}, υ_{mf}, k_{mg}, and k_{mf} to eqs 4 and 5 together with eq 7, one can easily evaluate the fractional conversion of glucose at any given time for various initial concentrations of glucose.

The conventional method reported in the literature for determining υ_{mg}, υ_{mf}, k_{mg}, and k_{mf} at a constant temperature is that experiments with feed solution containing either glucose or fructose should be carried out. By estimating the initial rates of the glucose to fructose and vice versa, these parameters can be determined using the Lineweaver−Menten equation.[20] However, one can evaluate these four key parameters (i.e., υ_{mg}, υ_{mf}, k_{mg}, and k_{mf}) by carrying out just experiments for glucose to fructose reaction. Camacho-Rubio et al. have rewritten the rate of reaction as follows:[21]

$$r_G = -\frac{1}{W}\frac{d[G]}{dt} = K_r\frac{(X_e - X)}{1 + KX} \tag{8}$$

with

$$K_r = \frac{\upsilon_{mg}(1 + K_e^{-1})}{k_{mg} + [G]_0}[G]_0 \tag{9}$$

and

$$K = \frac{[G]_0\left[\left(\frac{k_{mg}}{k_{mf}}\right) - 1\right]}{k_{mg} + [G]_0} \tag{10}$$

where K_e, W, and X denote the equilibrium constant, catalyst loading, and the fractional conversion of glucose, respectively. Integrating eq 8 gives the following relation:

$$t = \frac{[G]_0}{W}\frac{KX_e + 1}{K_r}\ln\left(\frac{X_e}{X_e - X}\right) - \frac{[G]_0}{W}\frac{K}{K_r}X \tag{11}$$

To evaluate K and K_r as a function of initial concentration of glucose (i.e., $[G_0]$), one can plot $tW/(X\,[G]_0)$ against $1/X\,\ln[X_e/(X_e - X)]$ for various constant initial concentrations. Note that the equilibrium fractional conversion of glucose (X_e) can be determined experimentally by conducting long enough experimental runs. On the other hand, the inversion of eqs 9 and 10 yields

$$\frac{1}{K_r} = \frac{k_{mg}}{\upsilon_{mg}\left(1 + \frac{1}{K_e}\right)}\frac{1}{[G]_0} + \frac{1}{\upsilon_{mg}\left(1 + \frac{1}{K_e}\right)} \tag{12}$$

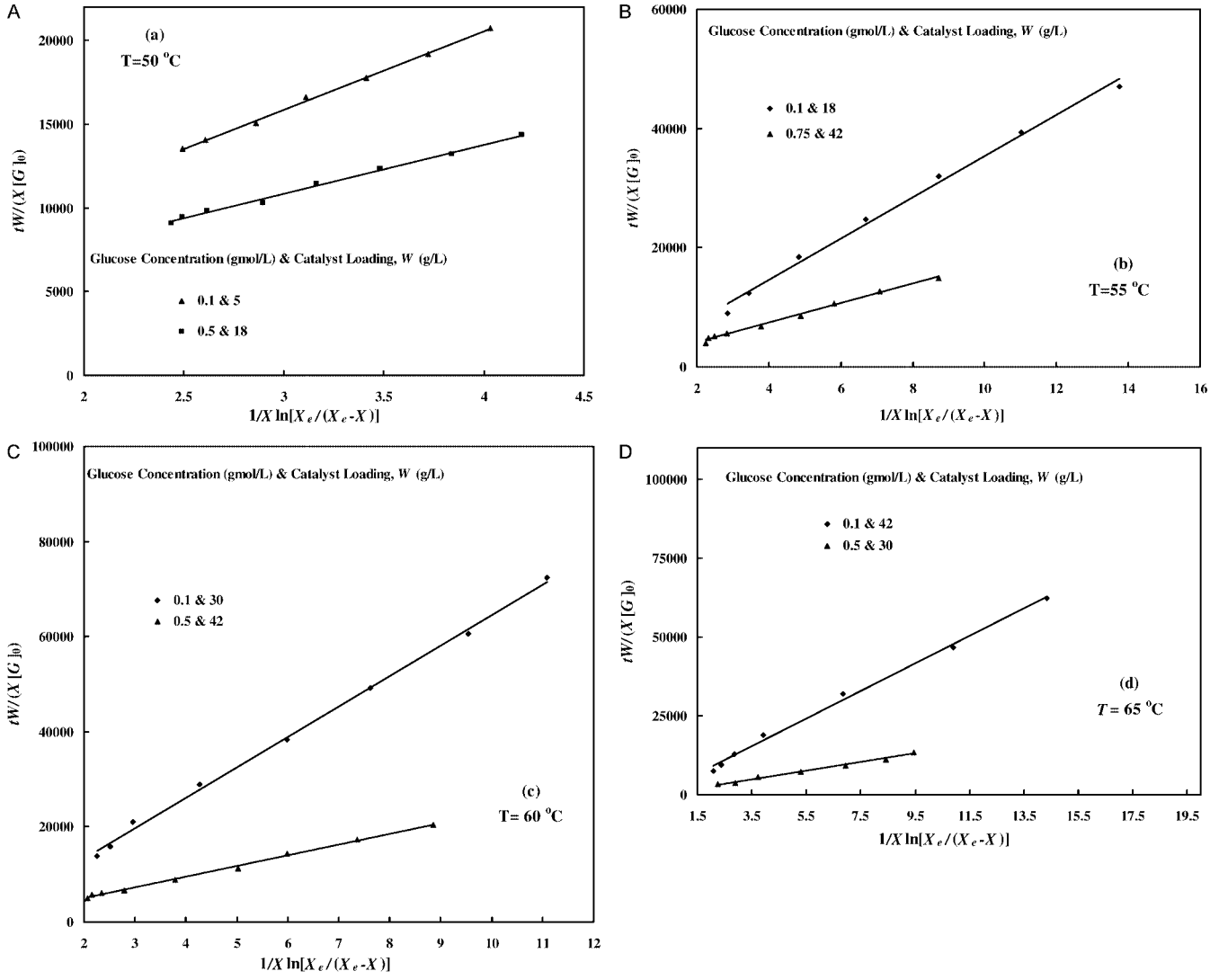

Figure 2. Variations of $tW/(X\,[G]_0)$ with $\ln[X_e/(X_e - X)]/X$.

$$\frac{1}{K} = \frac{k_{mg}}{\left[\left(\frac{k_{mg}}{k_{mf}}\right) - 1\right]}\frac{1}{[G]_0} + \frac{1}{\left[\left(\frac{k_{mg}}{k_{mf}}\right) - 1\right]} \quad (13)$$

Now, by plotting $1/K$ against $1/[G]_0$ one can easily evaluate k_{mg} and k_{mf}, and finally plotting $1/K_r$ against $1/[G]_0$, υ_{mg} could be determined. Having the value of K_e and using eq 6, υ_{mf} could be subsequently determined.

It should also be noticed that the kinetic parameters of the reaction (i.e., k_{mg}, k_{mf}, υ_{mf}, and υ_{mg}) are temperature dependent and their dependencies on the temperature can be given by Arrhenius-like expressions as follows:

$$k_{mg} = k_{mg0}e^{-E_{k_{mg}}/RT} \quad (14)$$

$$k_{mf} = k_{mf0}e^{-E_{k_{mf}}/RT} \quad (15)$$

$$\upsilon_{mg} = \upsilon_{mg0}e^{-E_{\upsilon_{mg}}/RT} \quad (16)$$

$$\upsilon_{mf} = \upsilon_{mf0}e^{-E_{\upsilon_{mf}}/RT} \quad (17)$$

The equilibrium constant of the isomerization reaction is also temperature dependent and can be expressed as

$$K_e = K_{e0}e^{-E_e/RT} \quad (18)$$

By evaluating the kinetic parameters of the reaction (i.e., k_{mg}, k_{mf}, υ_{mf}, and υ_{mg}) and the equilibrium constant of the isomerization reaction (i.e., K_e) at different temperatures based on the above-mentioned procedure and then plotting logarithmic values of the kinetic parameters (i.e., k_{mg}, k_{mf}, υ_{mf}, and υ_{mg}) vs $1/T$, one can easily determine the activation energies and frequency factors.

Results and Discussion

Evaluation of Kinetic Parameters. Figures 1a,b demonstrate typical experimental data regarding the kinetic behavior of glucose to fructose isomerization reaction. As may be noticed, slight deviations from the Michaelis−Menten kinetic model at low fractional conversions are observed which are shown by the rectangles. In fact, deviations from the Michaelis−Menten kinetic model at fractional conversions <10% is clearly visible. As fractional conversion increases, however, these deviations vanish and the fractional conversion curves perfectly match with the Michaelis−Menten kinetic model. This kind of deviation has been reported by Benaiges et al.[22] This behavior may be caused by a transitory behavior of the particles reflecting the time needed for the establishment of steady concentration profiles within the catalyst particles. These disturbances prevented us from relying on the initial rate of reaction to use Lineweaver−Menten method for estimating the kinetic parameters. Using the procedure mentioned earlier, one can easily omit these initial data points and estimate kinetic parameters using the higher conversion experimental data. Moreover, using the procedure explained in the experimental section, it is not necessary to conduct experimental runs with feed solutions containing only fructose to estimate υ_{mf} and k_{mf}. To determine the kinetic parameters K and K_r, the typical experimental data concerning $tW/(X[G]_0)$ were plotted against $1/X \ln[X_e/(X_e - X)]$ for each temperature as shown in Figure 2a−d. From these figures, the kinetic parameters K and K_r could be evaluated by linear regression analysis and the obtained results are summarized in Table 1. In addition, the equilibrium fractional conversion of glucose (X_e) was experimentally obtained and then the equilibrium constant of the reaction was calculated by eq 6. These results are summarized in Table 2.

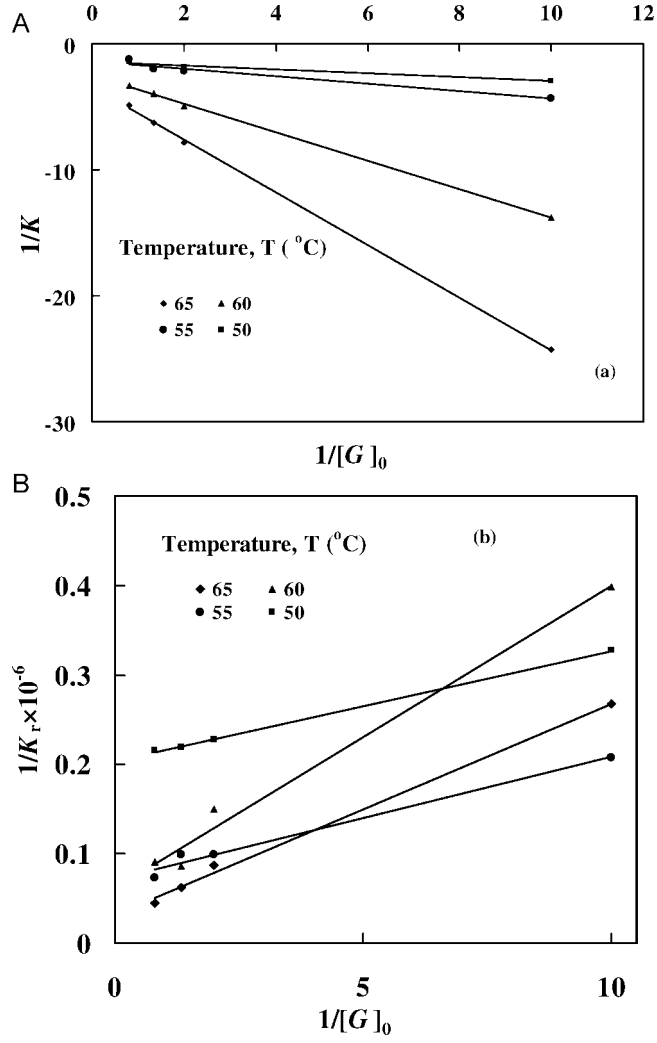

Figure 3. Variations of $1/K$ and $1/K_r$ with $1/[G]_0$.

With the obtained values of K and K_r at a constant temperature, one can easily evaluate the four key kinetic parameters, i.e., k_{mg}, k_{mf}, υ_{mg}, and υ_{mf}. To achieve this goal, the obtained values of $1/K$ and $1/K_r$ could be plotted against $1/[G]_0$ as shown in Figure 3a,b. Using the linear regression analysis, the four key kinetic parameters, i.e., k_{mg}, k_{mf}, υ_{mg}, and υ_{mf}, were found and are summarized in Table 2. By substituting the values of the four kinetic parameters (i.e., k_{mg}, k_{mf}, υ_{mg}, and υ_{mf}) into eqs 4 and 5, one can evaluate V_m and K_m at a given initial concentration of glucose $[G]_0$.

To show the goodness of the kinetic model parameters obtained by this procedure, the predicted fractional conversions of glucose using the obtained kinetic parameters are compared with the experimental data in Figures 4a−d and 5. As may be observed from these figures, there is a fair agreement between the experimental and predicted fractional conversions of glucose. In addition, to quantify the deviation between the predicted and experimental results, the root-mean-square (rms) of the normalized residuals was calculated according to the following expression:

$$\text{rms} = 100\sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(1 - \frac{X_i(\text{cal})}{X_i(\text{exp})}\right)^2} \quad (19)$$

where N is the number of experimental data points. The rms of the predicted values of fractional conversion of glucose was calculated to be 6.96%. This value of rms clearly shows the goodness of the kinetic model.

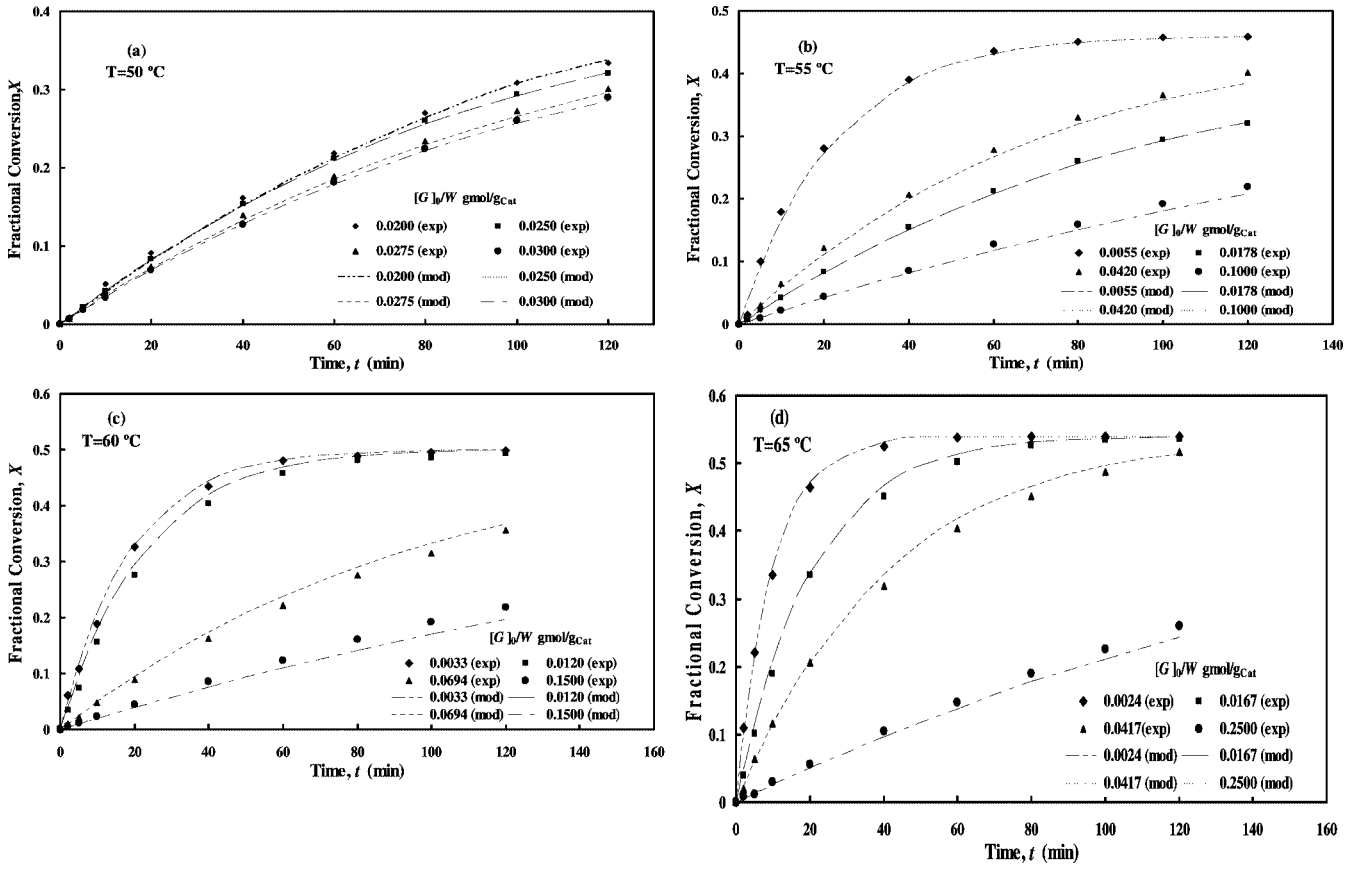

Figure 4. Variations of fractional conversion of glucose with time and comparison with the kinetic model predictions.

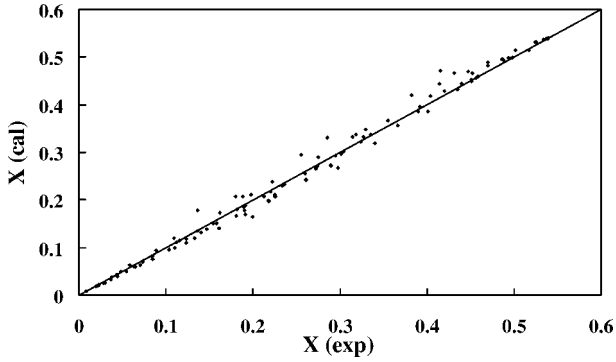

Figure 5. Comparison of predicted fractional conversion of glucose with experimental data.

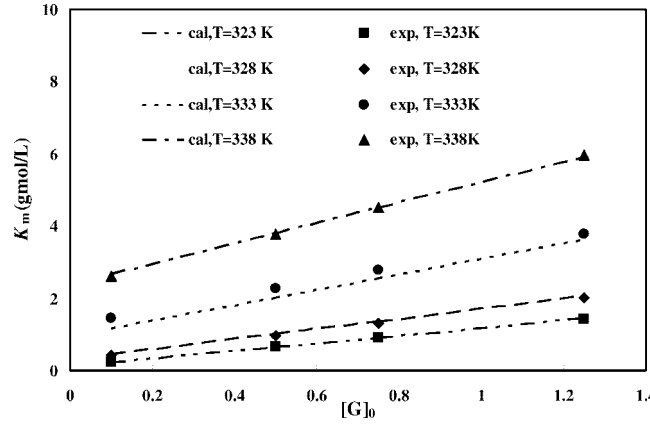

Figure 6. Variations of K_m with the initial concentration of glucose and comparison with the corresponding correlation in eq 20.

Temperature Dependence of the Kinetic Parameters. To explore the dependence of kinetic parameters (i.e., k_{mg}, k_{mf}, υ_{mg}, and υ_{mf}) and the equilibrium constant (K_e) on the reaction temperature, the obtained values of these kinetic parameters (i.e., k_{mg}, k_{mf}, υ_{mg}, and υ_{mf}) were plotted vs $1/T$. Based on this procedure, the frequency factors and the activation energies were evaluated, which are summarized in Table 3.

It is also possible to evaluate the temperature dependence of K_m and V_m and consequently the activation energies and frequency factors of K_m and V_m could be obtained. It should be noticed that the K_m is a function of $[G]_0$ and hence the activation energy and frequency factor of K_m vary with the initial concentration of glucose $[G]_0$. As expected from eq 4, at a constant temperature there is a linear relationship between K_m and $[G]_0$, whereas at a given initial concentration of glucose an Arrhenius-like expression can describe the variations of K_m with the reaction temperature. Therefore, by considering a linear function of K_m in terms of $[G]_0$ and the corresponding Arrhenius-like expression, it would be possible to derive a general correlation for K_m, by which the dependence of K_m on both the $[G]_0$ and T can be represented as follows:

$$K_m = 10.369\times10^{10}e^{-7534/T}[G]_0 + 5.479\times10^{32}e^{-22818/T} \quad (20)$$

where the calculated rms was 7.7%. Using eq 20, the values of K_m were estimated and were compared with those obtained with the obtained kinetic parameters, which are shown in Figure 6. Furthermore, the V_m is only dependent on the reaction temperature and can be given by

$$V_m = 8.814\times10^{20}e^{-19398/T} \quad (21)$$

where T and $[G]_0$ are in K and mol/m^3, respectively.

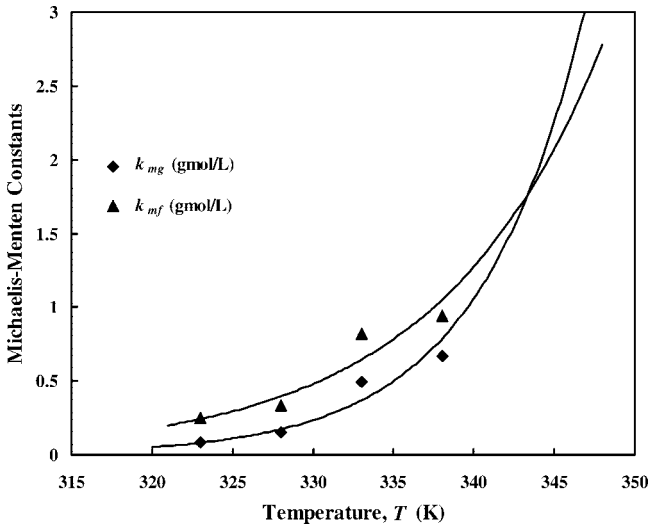

Figure 7. Variations of k_{mg} and k_{mf} with temperature.

The data presented in Table 2 show that for the reaction temperatures examined in the present work, the value of k_{mg}/k_{mf} ratio is smaller than unity. These small values of k_{mg}/k_{mf} ratio give negative values for K, and as it may be noticed from Table 2, the value of the ratio of k_{mg}/k_{mf} increases with increasing the reaction temperature. Therefore, it can be concluded that there is a temperature at which the k_{mg}/k_{mf} ratio becomes exactly unity.[12,23,24] This temperature is termed the characteristic temperature (T_L), which its value has been reported to be between 60 and 80 °C for other IGI catalysts. The characteristic temperature can be estimated using the correlations of k_{mg} and k_{mf}, which are shown in Figure 7 and can be expressed by

$$k_{mg} = 1.32 \times 10^{24} e^{-16524/T} \quad (22)$$

$$k_{mf} = 5.20 \times 10^{16} e^{-10661/T} \quad (23)$$

where T is in K. Using these correlations one can easily evaluate the characteristic temperature to be $T_L \cong 70$ °C. Note that, at this temperature, the value of K becomes zero and the reaction rate expression (i.e., eq 8) simplifies to the following linear rate expression

$$r_G = -\frac{1}{W}\frac{d[G]}{dt} = K_r(X_e - X) \quad (24)$$

The latter is a pseudo-first-order reaction rate expression, which has been reported in the literature.[3,14,25,26] Camacho-Rubio et al.[21] have collected the values of k_{mg} and k_{mf} reported in the literature for various types of IGI catalysts and concluded that these values are either equal or close to each other. Thus, they have suggested a pseudo-first-order reaction rate expression.

Note that over the reaction temperature range used in the present work, the obtained values of k_{mf} and k_{mg} are close to each other and hence a pseudo-first-order reaction rate expression may be applicable. By substituting K_r from eq 9 into eq 24 we get

$$r_G = -\frac{1}{W}\frac{d[G]}{dt} = \frac{\upsilon_{mg}(1 + K_e^{-1})}{k_{mg} + [G]_0}([G] - [G]_e) = K_L([G] - [G]_e) \quad (25)$$

with

$$K_L = \frac{\upsilon_{mg}(1 + K_e^{-1})}{k_{mg} + [G]_0} \quad (26)$$

It is obvious that if the isomerization reaction is carried out at the characteristic temperature (T_L), the reaction rate constant will only depend on the initial glucose concentration, $[G]_0$, and remains constant during the entire process. Therefore, the reaction rate expression would be a linear function of glucose concentration.[12]

As may be noticed from Table 2, the $\upsilon_{mg}/\upsilon_{mf}$ ratio is less than unity over the temperature range examined and increases with increasing the reaction temperature. Therefore, it could be concluded that there is a temperature at which the values of υ_{mg} and υ_{mf} become either equal or close to each other. This temperature can be estimated by using the correlations of υ_{mg} and υ_{mf}, which are presented in Figure 8, and can be expressed by

$$\upsilon_{mg} = 2.58 \times 10^{14} e^{-14932/T} \quad (27)$$

$$\upsilon_{mf} = 2.64 \times 10^{2} e^{-5555.2/T} \quad (28)$$

where T is in K. Considering these correlations, this temperature can be estimated to be ~67 °C. This temperature is about 3 °C less than 70 °C at which the values of k_{mg} and k_{mf} become either equal or close to each other. The linearity of the reaction rate expression at the T_L can also reasonably be assumed at

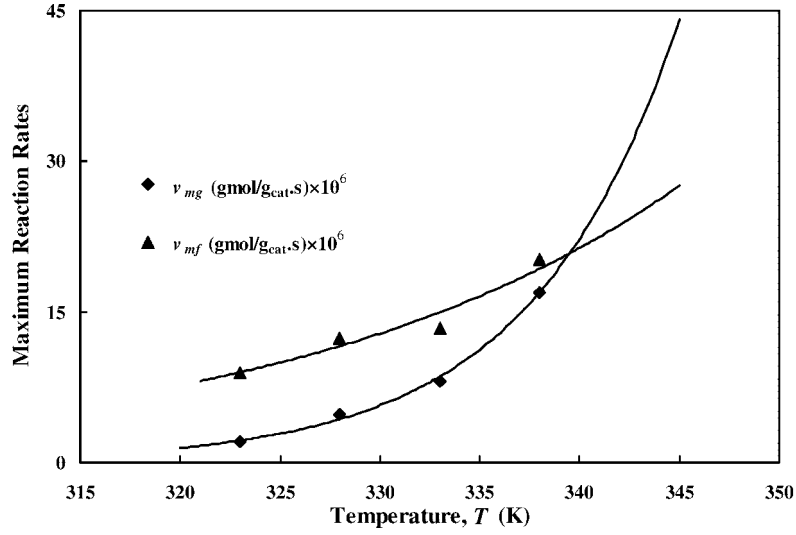

Figure 8. Variations of υ_{mg} and υ_{mf} with temperature.

temperatures close to T_L and so at 67 °C. The validity of this assumption has previously been verified by Palazzi and Converti.[12] At this lower temperature (i.e., $\leq T_L$) and using the linearized reaction rate expression along with the equal values of υ_{mf} and υ_{mg}, eq 24 reduces to the following rate expression

$$r_G = -\frac{1}{W}\frac{d[G]}{dt} = K_r\frac{(X_e - X)}{1 + KX} = \frac{2\upsilon_{mg}}{k_{mg} + [G]_0}([G] - [G]_e) \quad (29)$$

According to our calculations, for the reaction temperatures ranging from 65 to 75 °C, the product of $KX \ll 1$ and hence we can use the above linear reaction rate expression. In fact, within a specific range of temperature, one can approximately express the rate of the reversible reaction as an irreversible reaction by considering only the kinetic parameters of glucose to fructose reaction and neglecting the kinetic parameters of the reverse reaction.

Conclusions

An experimental and theoretical investigation was conducted on the kinetic modeling of isomerization reaction of D-glucose to D-fructose by the commercial immobilized glucose isomerase, Sweetzyme IT. The kinetic parameters of the isomerization reaction were determined through experimental data analysis, employing the Michaelis−Menten kinetic model, and using the developed procedure. From experimental data, the temperature dependence of the kinetic parameters and equilibrium constant were evaluated and correlated. It was found as follows:

(1) There are slight deviations from the Michaelis−Menten kinetic model at low fractional conversions. As fractional conversion increases, however, these deviations vanish and the fractional conversion curves perfectly match with the Michaelis−Menten kinetic model.

(2) The above-mentioned deviations prevent one from relying on the initial rate of reaction to use Lineweaver−Menten method for estimating the kinetic parameters of the glucose to fructose isomerization.

(3) By considering a linear function of K_m in terms of the $[G]_0$ and using an Arrhenius-like expression, a general correlation for K_m was obtained in order to express its dependence on both the $[G]_0$ and T.

(4) The characteristic temperature was estimated using the correlations obtained for k_{mg} and k_{mf}. At this characteristic temperature, the value of K becomes zero and the reaction rate expression reduces to a simple linear rate expression.

Acknowledgment

The authors gratefully acknowledge Novo Nordisk (Iran) for providing IGI catalyst and Sharif University of Technology for providing financial support.

Appendix

Nomenclature

E = activation energy (J/mol)
En = enzyme (−)
$[F]$ = concentration of fructose (mol/m^3)
$[F]_e$ = equilibrium concentration of fructose (mol/m^3)
$[G]$ = concentration of glucose (mol/m^3)
$[G]_e$ = equilibrium concentration of glucose (mol/m^3)
$[G]_0$ = initial concentration of glucose (mol/m^3)
$[\bar{G}]$ = concentration of glucose in eq 3 (mol/m^3)
GEn = intermediate complex (−)
K = kinetic constant in eq 10 (−)
K_e = equilibrium constant in eq 6 (−)
K_m = apparent Michaelis−Menten constant in eq 4 (mol/m^3)
K_r = kinetic constant in eq 9 (mol/(g$_{cat}$·s))
k_{mf} = Michaelis−Menten constant for fructose (mol/m^3)
k_{mg} = Michaelis−Menten constant for glucose (mol/m^3)
K_{e0} = frequency factor of equilibrium constant (−)
k_{mf0} = frequency factor of k_{mf} (mol/m^3)
k_{mg0} = frequency factor of k_{mg} (mol/m^3)
R = universal gas constant (J/(mol·K))
r_G = reaction rate (mol/(g$_{cat}$·s))
T = temperature (K)
t = time (s)
V_m = maximum apparent reaction rate (mol/(g$_{cat}$·s))
υ_{mf} = maximum apparent reaction rate for fructose (mol/(g$_{cat}$·s))
υ_{mg} = maximum apparent reaction rate for glucose (mol/g$_{cat}$·s)
υ_{mf0} = frequency factor of υ_{mf} (mol/(g$_{cat}$.s))
υ_{mg0} = frequency factor of υ_{mg} (mol/(g$_{cat}$·s))
W = catalyst loading (g/L)
X = fractional conversion of glucose (−)
X_e = equilibrium fractional conversion of glucose (−)

Abbreviations

cal = calculated
cat = catalyst
exp = experimental
mod = model

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Received for review March 11, 2008
Revised manuscript received December 23, 2008
Accepted January 13, 2009

IE800400B