Kinetic Study of Fructose-Glucose Isomerization in a Recirculation Reactor

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We have studied the equilibrium and kinetics of fructose-to-glucose isomerization using a commercial immobilized glucose isomerase, Sweetzyme T® (from NOVO), in a packed-bed recirculation reactor which enabled us to eliminate the influence of external-mass transfer and which could function as a differential reactor when a sufficient flow rate was used. The results obtained have also been corrected with regard to the influence of internal transport in the immobilized enzyme particles.

Isomerization kinetics is a pseudo first-order reversible reaction in the ranges studied: 303–333 *K*, pH 7.5 and 0.5 to 2.0 mol/L hexose concentrations. We compare our results with those found in the literature, and propose equations for the influence of temperature on the equilibrium and on the kinetic constants.

Nous avons étudié l'équilibre et la cinétique de l'isomérisation du fructose en glucose à l'aide d'une isomérase de glucose immobilisée commerciale, Sweetzyme T® (de NOVO), dans un réacteur à recirculation à lit garni; ce réacteur nous a permis d'éliminer l'influence du transfert de matière externe et pouvait fonctionner comme réacteur différential si on appliquait un débit suffisant. Les résultats obtenus ont également été corrigés en ce qui concerne l'influence du transport interne dans les particules d'enzyme immobilisées.

La cinétique d'isomérisation est une réaction réversible du pseudo-premier order dans les gammes étudiées, à savoir: 303-333 *K*, pH 7,5 et concentration d'hexose de 0,5 à 2,0 mol/L. Nous avons comparé nos résultats avec ceux de la littérature scientifique et nous proposons des équations pour l'influence de la température sur l'équilibre et les constantes cinétiques.

Keywords: recirculation reactor, fructose, glucose, isomerization equilibrium.

The commercial applications of immobilized enzymes are of increasing interest, both in the food industry and in the synthesis of numerous compounds (Vos et al., 1990). One of the most important commercial applications is the use of glucose isomerase in obtaining syrups rich in fructose, for the sweetener market and for the manufacture of dietary foodstuffs. However, the kinetic studies of this system generally use initial-reaction rates and report highly contradictory results, mainly regarding the kinetic constants and the influence of internal transport in the immobilized enzyme particles.

In kinetic studies with immobilized enzymes, it is essential to have a reactor system free from the limitations of external mass transfer to the particles so as to approach the ideal flow patterns of plug flow or perfect mixing. For this work, a fixed-bed recirculation reactor has been developed to allow the easy detection of the effects of external-mass transfer and the mixing level on the reaction rate when immobilized enzymes are used.

Kinetic model

Glucose-fructose enzymatic isomerization is a reversible reaction, which appears to take place via the following mechanism:

$$G + E \underset{k_g}{\overset{k_{-g}}{\rightleftharpoons}} \times \underset{k_f}{\overset{k_{-f}}{\rightleftharpoons}} F + E \quad \ldots\ldots (1)$$

where G represents glucose, E the enzyme, F fructose and X the intermediate complex. Mechanism (1) yields the following expression for the net velocity:

$$r_{FG} = \frac{(V_{mf}/K_{mf})\ ([F] - K_e\ [G])}{1 + ([F]/K_{mf}) + ([G]/K_{mg})} \quad \ldots\ldots (2)$$

where

$$K_{mf} = (k_{-f} + k_{-g})/k_f \quad \ldots\ldots (3)$$

$$K_{mg} = (k_{-f} + k_{-g})/k_g \quad \ldots\ldots (4)$$

$$V_{mf} = k_{-g}\ e_t \quad \ldots\ldots (5)$$

$$K_e = \frac{k_{-f}\,k_g}{k_f\,k_{-g}} = \left(\frac{[F]}{[G]}\right)_{eq} \quad \ldots\ldots (6)$$

where e_t represents the total moles of active enzyme per kg of enzymatic complex used.

Let us consider the fructose → glucose transformation, introducing the conversion, x, referring to the total hexose concentration, s_o, and defined as

$$x = [G]/s_o \quad \ldots\ldots (7)$$

From Equation (2), we get

$$r_{FG} = \frac{V_{mf}\,s_o\,(1 + K_e)\,(x_e - x)/K_{mf}}{(1 + s_o/K_{mf}) + [(s_o/K_{mg}) - (s_o/K_{mf})]\,x}$$

$$= k_r \frac{(x_e - x)}{1 + K\,x} \quad \ldots\ldots (8)$$

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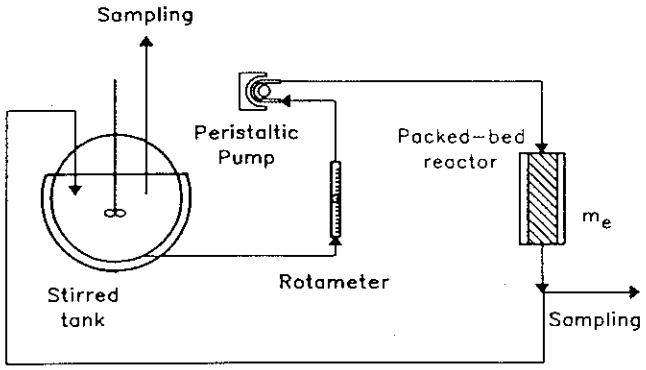

Figure 1 — Recirculation reactor system.

where

$$k_r = \frac{V_{mf}\,(1 + K_e)}{K_{mf} + s_o}\, s_o \quad \text{..... (9)}$$

$$K = \frac{s_o\,(K_{mf}/K_{mg}) - 1)}{K_{mf} + s_o} \quad \text{..... (10)}$$

Materials and methods

The substrate used was $D(-)$ fructose (Merck, Darmstadt, Germany) and the enzyme Sweetzyme T (Novo Industries, Bagsvaerd, Denmark), obtained from a selected strain of Streptomyces murinus.

The experimental apparatus (Figure 1), consists of a 0.25 L capacity jacketed tank, intensely stirred, from which the load is extracted using a peristaltic pump and made to flow through a cylindrical jacketed packed-bed reactor, 2.5 cm internal diameter and 9.0 cm high, within which the immobilized enzyme is confined between two porous glass sheets. The reactor effluent is recirculated to the tank.

The fructose load was prepared by dissolving the necessary quantity of fructose in a solution of $MgSO_4 \cdot 7H_2O$ of 0.55 kg/m^3 (to stabilize the enzyme), and the pH was adjusted to 7.5 with a 100 mol/m^3 solution of Na_2CO_3.

The kinetics of the fructose → glucose reaction was monitored by taking time-related samples from the tank and determining the glucose concentration using the glucose-oxidase method proposed by Werner et al. (1970).

The enzymatic activity, defined as moles of glucose produced per second per kg of enzyme at 323 K, proved to be $1.82 \cdot 10^{-3}$. We experimentally verified that no active enzyme was transported from the bed to the tank, and during the first 24 hours of use no activity loss was perceived.

Isomerization-equilibrium experiments were performed at a total hexose concentration of 2.0 mol/m^3. The reaction, pH 7.5 and temperatures of 303 to 333 K, was studied in both directions.

Results and discussion

Reactor model

If the packed-bed reactor can be considered to be differential, a glucose balance on this reactor and another on the whole system yield:

$$m_e/q = s_o\,(x_s - x)/r_{FG} \quad \text{..... (11)}$$

$$V\,dx/dt = q\,(x_s - x) \quad \text{..... (12)}$$

where m_e is the mass of immobilized enzyme in the packed-bed reactor in kg, q is the flow rate in m^3/s and V the total liquid phase volume in m^3.

Combining Equations (11) and (12), we obtain

$$dx/dt = m_e\, r_{FG}/s_o\, V \quad \text{..... (13)}$$

This equation shows that when the conversion variation over time in the system is independent of q, r_{FG} is not influenced by external-mass transfer to the catalyst particles, and the reactor functions as a differential one, without the mixing level in the bed having any influence. At this stage, the results should be equivalent to those obtained in a perfectly mixed reactor.

To obtain these conditions, we carried out a series of experiments with 1000 mol/m^3 fructose concentration, 5.0 g of immobilized enzyme in the bed, at 323 K and pH 7.5, modifying the flow rate from 1.83 to 9.72 mL/s. The results indicate that for the range of flow rates used, the conversion is independent of q. In all subsequent experiments, a flow rate of 3.17 mL/s was used.

Since, during the experiments, samples were taken from the system for analysis, the total volume of solution contained in the system changed gradually; thus, the following equation has been used as an intensive treatment variable (Jurado et al., 1994) to accommodate this circumstance:

$$y = m_e\, \Sigma\, \Delta t_i/V_i \quad \text{..... (14)}$$

where V_i is the volume of the liquid phase during the time, t_i, and Δt_i represents the time increment between the two consecutive samplings.

Bearing in mind Equations (8) and (14) and substituting in Equation (13), we get

$$dx/dy = k_a\,(x_e - x)/(1 + K\,x) \quad \text{..... (15)}$$

where

$$k_a = k_r/s_o \quad \text{..... (16)}$$

Before applying this equation to the kinetic experiments, it is useful to determine the equilibrium conversion.

Isomerization equilibrium

The equilibrium conditions in the temperature range 303 to 333 K have been determined by conducting two experiments with the same total hexose concentration for each temperature, using fructose and glucose mixtures with concentrations close to the equilibrium on either side, and following the isomerization until the glucose concentration remained practically constant.

Figure 2 shows the K_e values obtained, together with values found in the literature for this constant versus the inverse of the absolute temperature. This figure indicates that the data fit the Van't Hoff equation. By linear regression, excluding only those data that depart from the resulting equation by more than 5%, we obtain

$$K_e = 24.3 \exp\,(-1022/T) \quad \text{..... (17)}$$

an equation which is also represented in Figure 2 in the form of a continuous line.

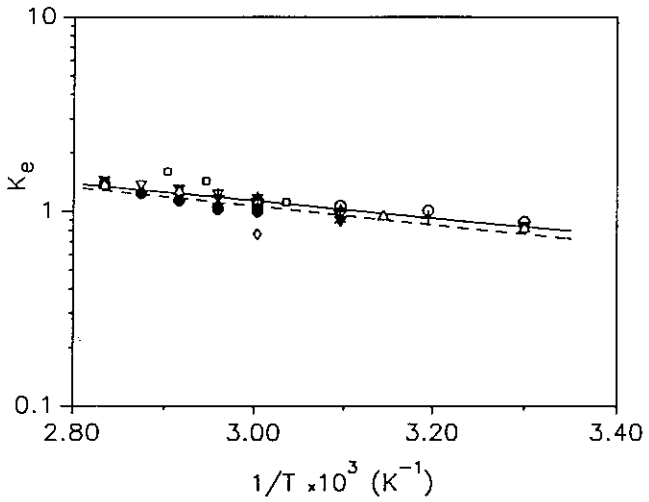

Figure 2 — Van't Hoff plot of equilibrium constants. +, Takasaki et al. (1969); Δ, Sproull et al. (1976); □, Lee et al. (1976); ▼, Lloyd and Khaleeluddin (1976); X, Kikkert et al. (1981); ⊡, Park et al. (1981); ▽, Chen et al. (1983); ◇, Benaiges et al. (1986); ●, Chen and Wu (1987); ■, Visuri and Klivanov (1987); ▲, Gram et al. (1990); ○, Present Study; ——— Roels and Van Tilberg (1979) equation; ——— Equation (17).

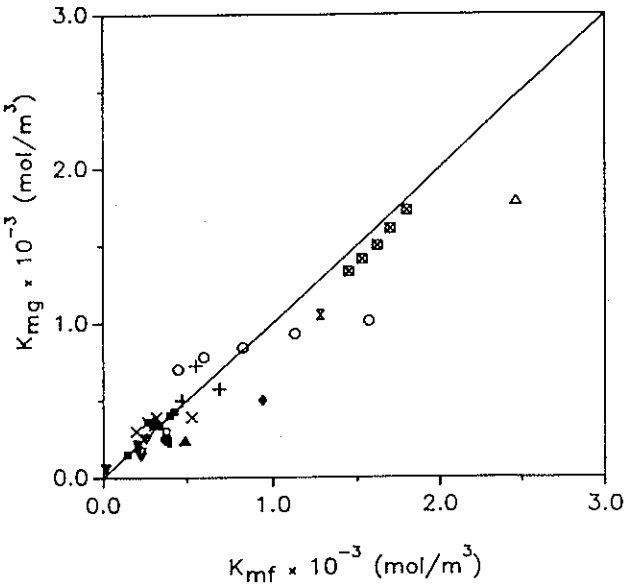

Figure 3 — Michaelis-Menten constant for glucose, K_{mg}, versus Michaelis-Menten constant for fructose, K_{mf}. ▼, Takasaki et al. (1969); ■, Krasnobaev and Böeniger (1975); ●, Saini and Vieth (1975); □, Lee et al. (1976); ▽, Lloyd and Khaleeluddin (1976); +, Sproull et al. (1976); ▲, Ryu et al. (1977); ⊠, Chen et al. (1980); ◇, Adachi et al. (1981); X, Kikkert et al. (1981); ◆, Standard Brands (taken of Kikkert et al., 1981); ⊡, Van Keulen et al. (1981); △, Chen et al. (1983); ⧗, Ching and Ho (1984); ○, Chen and Wu (1987); □, Present study.

ISOMERIZATION KINETICS

The integration of Equation (15) gives

$$y = \frac{1}{k_a} \ln\left(\frac{x_e - x_o}{x_e - x}\right) + \frac{K}{k_a}(x - x_o) \quad \text{..... (18)}$$

A comparison of this equation with the experimental results indicates that the value of K is practically zero, and thus $K_{mf} = K_{mg}$. Equation (18) is therefore reduced to

$$y = \frac{1}{k_a} \ln \frac{x_e - x_o}{x_e - x} \quad \text{..... (19)}$$

which corresponds to the kinetics of a pseudo first-order reaction (Straatsma et al., 1983; Nakamura et al., 1984; Vos et al., 1990).

The values of the constants, K_{mf} and K_{mg}, found in the literature, and shown in Figure 3, are invariably close and vary randomly regardless of the operating conditions under which the values have been determined (T and pH, the enzymatic complex used). Experimental results largely correspond to values that are equal or close to both constants.

Equation (19) has been applied to all the results obtained, verifying that it fulfills its function acceptably. Figure 4, for example, shows the data obtained at 323 K, pH 7.5 and a substrate concentration of 1000 mol/m^3, varying the enzyme mass in the reactor. All fit a single straight line, as might be expected.

Non-linear regression of the experimental results to Equation (19) gives the values of the pseudo first-order rate constants shown in Table 1. This kinetic constant should include the effectiveness factor if the internal transport in the catalyst particles influences the overall velocity of the process.

INFLUENCE OF INTERNAL TRANSPORT

Since the reaction rate fits a pseudo first-order equation, the effectiveness factor, η, will depend solely on the Thiele modulus, ϕ, which, for spherical particles, is given by the expressions

$$\phi = (d_p/6)\ [\rho_p\ k/D_e]^{0.5} \quad \text{..... (20)}$$

and

$$\eta = \frac{1}{\phi}\left[\frac{1}{\tanh(3\phi)} - \frac{1}{3\phi}\right] \quad \text{..... (21)}$$

the previously determined kinetic constant being

$$k_a = \eta\ k \quad \text{..... (22)}$$

Equations (20)–(22) can enable us to calculate the kinetic constant k, without the effects of internal transport, whenever we know the diameter of the catalyst particles, d_p, the apparent density of these particles, ρ_p and the effective diffusivity, D_e. The average particle size and the apparent density of the particles have been determined by means of mercury intrusion and correspond to $d_p = 0.65$ mm and $\rho_p = 1310$ kg/m^3.

In general, the effective diffusivity has been related to molecular diffusivity by means of the expression

$$D_e = D\ \epsilon/\tau \quad \text{..... (23)}$$

In the above equation, D is the fructose molecular diffusivity (6.9×10^{-10} m^2/s; $T = 50$°C; $s_o = 0.5$ mol/L, Lee et al., 1976), ϵ represents particle porosity and τ the

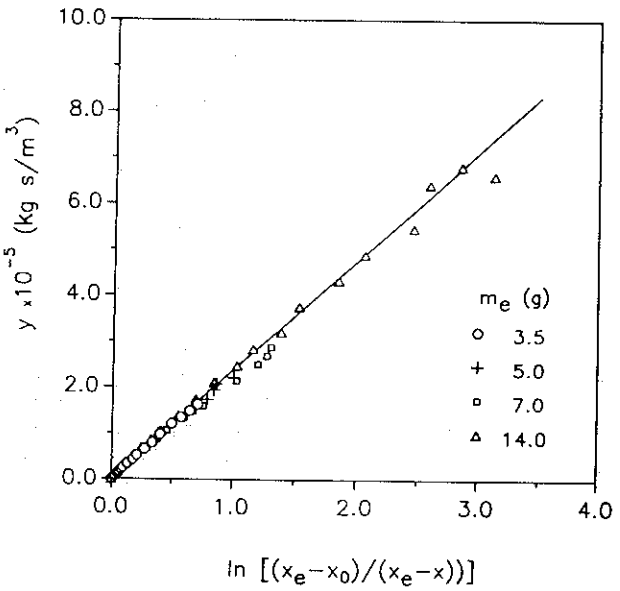

Figure 4 — Plot of Equation (19). T = 323 K; pH = 7.5; s_o = 1.0 mol/L.

TABLE 1
Values for k_a and k, Thiele Modulus and Effectiveness Factor

T (K)	s_o (mol/m^3)	k_a (m^3/kg s)×10^6	ϕ	η	k (m^3/kg·s)×10^6
303	500	1.3	0.32	0.94	1.3
	1000	0.81	0.29	0.95	0.84
	2000	0.40	0.28	0.96	0.42
313	500	3.3	0.48	0.88	3.7
	1000	1.9	0.41	0.91	2.1
	2000	1.1	0.42	0.91	1.2
323	500	6.8	0.65	0.82	8.3
	1000	4.2	0.57	0.85	5.0
	1500	3.4	0.60	0.84	4.1
	2000	2.5	0.61	0.83	3.0
333	500	14	0.93	0.70	21
	1000	8.4	0.77	0.76	11
	2000	5.0	0.83	0.74	6.8

tortuosity factor which generally varies between 3 and 5 for a porous carrier particle.

Nevertheless, using stimulus-response experiments, Nakamura et al. (1984) directly measured D_e in immobilized glucose isomerase particles which were very similar to those used in the present study. These authors found the effective diffusivity at 60% of the molecular diffusivity, a percentage that roughtly coincides with the porosity of their particles. Chen et al. (1980) and Houng et al. (1993) achieved comparable results using a commercial glucose-isomerase immobilized in the gel matrix.

To explain these results, we should bear in mind that a particle formed by a gel matrix containing small pockets of an aqueous enzyme solution differs markedly from a porous carrier particle with an active catalytic agent or an enzyme linked to the inner surface of the pores. The immobilized enzyme is free in the pockets contained in the gel matrix, and the chemical reaction occurs only in these pockets when reached by the substrate molecules, while the substrate and the product diffuse through the microporous structure of the gel without a chemical reaction.

Taking into account the above results concerning enzymes occluded in the gel matrix, we have used the following equation to evaluate effective diffusivity:

$$D_e = \epsilon D \quad \text{..... (24)}$$

where the porosity of the particle is 45%.

Equations (20)–(22) enable us to determine the values of ϕ, η and k from the results obtained previously (Table 1).

DETERMINING THE KINETIC CONSTANTS

Table 1 shows that at each temperature the k values drop when the substrate concentration is increased, as might be expected from Equation (9). By a linear regression of these results, the values of V_{mf} and K_{mf} are obtained (Table 2).

Using the values of the equilibrium constant determined previously, together with those of K_{mf} and V_{mf}, we have obtained the values of the kinetic constants of the proposed model, Equation (1), all of these including the value of e_t (Table 2).

TABLE 2
Rate Constants of the Isomerization Fructose-Glucose

T (K)	V_{mf} (mol/kg·s)×10^4	$K_{mf} = K_{mg}$ (mol/m^3)	$k_f e_t = k_g e_t$ (m^3/kg·s)×10^6	$k_{-f} e_t$ (mol/kg·s)×10^4	$k_{-g} e_t$ (mol/kg·s)×10^4
303	4.9	137	6.6	4.1	4.9
313	13	190	13	12	13
323	37	420	18	38	37
333	73	310	49	82	73

TABLE 3
Activation Energies for Rate Constants

E_{ag} (kJ/mol)	E_{af} (kJ/mol)	E_{a-g} (kJ/mol)	E_{a-f} (kJ/mol)	Kind of system	Refs.
68.5	72.7	81.9	92.0	Whole cells.	Sproull et al. (1976)
67.27	64.90	49.88	58.20	Immobilized whole cells	Chen et al. (1980)
40.00	35.00	55.00	55.00	Immobilized enzyme	Kikkert et al. (1981)
11.00	54.57	59.49	86.94	Immobilized enzyme	Chen and Wu (1987)
53	53	76	85	Immobilized enzyme	Present study

Applying the Arrhenius equation, we obtain

$$k_{-f}\, e_t = 1.7 \times 10^{11} \exp(-10000/T) \quad \ldots\ldots\ldots\ldots (25)$$

$$k_{-g}\, e_t = 7.2 \times 10^{9} \exp(-9200/T) \quad \ldots\ldots\ldots\ldots (26)$$

$$k_f\, e_t = k_g\, e_t = 7.7 \times 10^{3} \exp(-6300/T) \quad \ldots\ldots\ldots (27)$$

The activation energies deduced from these equations can be compared to the values found in the literature (Table 3).

The values we obtained for the activation energies of the decomposition constants of the intermediate complex acceptably coincide with those of Sproull et al. (1976). Given that these authors worked with free whole cells containing glucose isomerase, this concordance signifies that the hypotheses proposed for effective diffusivity are acceptable.

In addition, if the influence of the internal transport is underestimated, values for apparent activation energies should be lower than real values. Therefore, if the activation energies calculated for the kinetic constants are of the same order of magnitude or greater than published values, then the evaluation of the internal transport influence would be accurate.

Nomenclature

d_p = particle diameter, m
D = molecular diffusion coefficient, m^2/s
D_e = effective diffusion coefficient, m^2/s
e_t = concentration of glucose isomerase per unit mass of immobilized enzyme, mol/kg
E_i = activation energy for k_i, kJ/mol
$[F]$ = fructose concentration, mol/m^3
$[G]$ = glucose concentration, mol/mol^3
k = pseudo first-order rate constant, $m^3/kg \cdot s$
k_a = pseudo first-order rate constant with the effects of internal transport, $m^3/kg \cdot s$
k_f, k_g = rate constants, Equation (1), $m^3/mol \cdot s$
k_{-f}, k_{-g} = rate constants, Equation (1), s^{-1}
k_r = kinetic constant, Equation (9)
K = kinetic constant, Equation (10)
K_e = equilibrium constant
K_{mf} = Michaelis-Menten constant for fructose, mol/m^3
K_{mg} = Michaelis-Menten constant for gloucose, mol/m^3
m_e = mass of immobilized enzyme, kg
q = flow rate, m^3/s
r_{FG} = reaction rate of the enzymatic fructose → glucose reaction, $mol/kg \cdot s$
s_o = initial concentration of substrate, mol/m^3
t = time of reaction, s
T = temperature, K
V = volume of reaction medium, m^3
V_{mf} = maximum reaction rate of the fructose, $mol/s \cdot kg$
x = conversion
x_e = equilibrium conversion
x_o = initial conversion
x_s = exit reactor conversion
y = intensive variable of the treatment, $kg \cdot s/m^3$

Greek letters

ϵ = porosity of the particle
η = effectiveness factor of the internal transport
ϕ = Thiele modulus
ρ_p = apparent density of the particle, kg/m^3
τ = tortuosity factor

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