Generalized Linearization of Kinetics of Glucose Isomerization to Fructose by Immobilized Glucose Isomerase

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Abstract: The kinetic parameters of both glucose isomerization to fructose and immobilized glucose isomerase (GI) inactivation calculated under different conditions are compared and discussed. Utilizing these figures, the possibility of generalizing a linear model, previously proposed for the kinetics of glucose isomerization by immobilized glucose isomerase, is investigated, so as to apply them to whole ranges of temperature and concentrations of actual interest in industrial processes. The proposed model is a satisfactory approximation of the more involved Briggs–Haldane approach and substantially simplifies the problem of optimizing an industrial fixed-bed column for high-fructose corn syrup (HFCS) production. © 1999 John Wiley & Sons, Inc. *Biotechnol Bioeng* **63**: 273–284, 1999.

Keywords: glucose isomerization; glucose isomerase; kinetics; enzyme inactivation; substrate protection

INTRODUCTION

The increased interest in high-fructose corn syrup (HFCS) is demonstrated by the fact that the U.S. market of sweetener is dominated by it, and even in Europe its consumption is rapidly growing (Riscolo and Fisichella, 1996). These alternative sweeteners, which contain no less than 42% fructose (Rechigl, 1982), are produced in Europe predominantly by continuous isomerization of concentrated starchy glucose syrups in immobilized GI columns.

Column performance is affected by thermal inactivaton of the enzyme (Illanes et al., 1996; van den Tweel et al., 1993) and diffusion resistance (Chen and Chang, 1984), whereas the presence of substrate proves to protect the enzyme, probably by stabilization of the activated complex tertiary structure induced by the link between active site and glucose (Verhoff and Goldstein, 1982). The conventional reversible Briggs–Haldane mechanism proves to be the best approach to describe isomerization kinetics, coupled with the activity decrease of the complexed GI, in both suspended (Roels, 1983) and immobilized systems (Chen and Wu, 1987), in either the presence or absence of substrate (Converti and Del Borghi, 1997, 1998).

This inactivation, primarily thermal inactivation, would be responsible for a progressive decrease in the isomerization yield and thus a poor quality product, if a traditional continuous process is carried out. As consistency of product composition is one of the main requirements for industrial application, much effort has been devoted to the search for a process capable of ensuring a constant fructose yield (Abu-Reesh and Faqir, 1996; Converti et al., 1997; Houng et al., 1993; Illanes et al., 1992).

The complexity of the kinetic model makes it impossible to obtain, in analytical form, the concentration distribution along the reactor as well as inside the catalyst pellet, so therefore reactor performance can be achieved only by numerical methods (Verhoff and Goldstein, 1982), which hardly elucidate the role of the most significant variables in process optimization. From the results of batch tests carried out within a starting glucose concentration range of practical significance ($500 \leqslant G_o \leqslant 3000$ mol m^{-3}) and at a temperature ($T = 75$°C) very close to the optimum, a recent study demonstrated that the isomerization rate can be linearized satisfactorily with respect to substrate concentration (Palazzi and Converti, 1997).

Linearity is examined in this study using wide ranges of temperature and glucose concentration in the feed. Moreover, the protection action of substrate on enzyme stability is shown to depend only on the total sugar concentration, being nearly constant with varying either temperature or glucose concentration.

THEORY

Reaction Kinetics

In consideration of the progressive thermal deactivation of the enzyme, the actual rate, v, of glucose isomerization to fructose in a reactor containing a biocatalyst depends on the specific isomerization rate of the fresh catalyst, v', and its residual activity, ψ, according to:

$$v = \psi\, v' \tag{1}$$

with ψ being the ratio of the actual concentration of the active enzyme, E_t, to that of the fresh enzyme, $E_{t,o}$:

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$$\psi = E_t/E_{t,o} \tag{2}$$

Glucose Isomerization Kinetics

The kinetics of some reversible reactions catalyzed by enzymes, such as the glucose isomerization to fructose, can be described by the Briggs–Haldane mechanism (Chen and Wu, 1987; Roels, 1983):

$$\mathrm{G} + \mathrm{E} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \mathrm{X} \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} \mathrm{F} + \mathrm{E} \tag{3}$$

where E is the active free enzyme, G is glucose, F is fructose, and X is the intermediate complex between the enzyme and glucose (EG) or fructose (EF), respectively.

Let k_{+1}, k_{-1}, k_{+2}, and k_{-2} be the rate constants of the elementary reactions and G the actual glucose concentration. Applying the pseudo-steady-state hypothesis for X, the Briggs–Haldane approach leads to the Michaelis–Menten-type equation:

$$v' = \frac{v'_m(G - G_e)}{K_m + (G - G_e)} \tag{4}$$

where G_e is the glucose concentration in equilibrium with that in the feed, G_o:

$$G_e = \frac{G_o}{1 + K} \tag{5}$$

and K is the equilibrium constant, defined as:

$$K = \frac{v'_{mf}k_{mr}}{v'_{mr}k_{mf}} \tag{6}$$

Parameters v'_m and K_m, appearing in Eq. (4), are, on the other hand, the analogous of maximum reaction rate and Michaelis constant for the whole isomerization:

$$v'_m = [1 + (1/K)]\frac{k_{mr}v'_{mf}}{k_{mr} - k_{mf}} \tag{7}$$

$$K_m = \frac{k_{mf}k_{mr}}{k_{mr} - k_{mf}}\left[1 + \left(\frac{1}{k_{mf}} + \frac{K}{k_{mr}}\right)G_e\right] \tag{8}$$

where:

$$k_{mf} = (k_{-1} + k_{+2})/k_{+1} \tag{9}$$

$$k_{mr} = (k_{-1} + k_{+2})/k_{-2} \tag{10}$$

$$v'_{mf} = k_{+2}\,E_t \tag{11}$$

$$v'_{mr} = k_{-1}\,E_t \tag{12}$$

are the Michaelis constants and the maximum velocities of the forward and the reverse reactions, respectively.

It is convenient to rewrite Eq. (4) in the form:

$$v' = k_i\,(G - G_e) \tag{13}$$

Parameter k_i, which essentially represents a kinetic constant for the isomerization, and is in general a function of G, G_o, and T, can be split up as:

$$k_i = k'_i\,(T)\,k''_i\,(G, G_o, T) \tag{14}$$

where:

$$k'_i = \frac{K + 1}{K}\frac{v'_{mf}}{k_{mf}} \tag{15}$$

$$k''_i = \frac{1}{1 + \lambda G_o} \tag{16}$$

$$\lambda = k_{mf}^{-1} - \Delta[1 - (G/G_o)] = \lambda\,(G, G_o, T) \tag{17}$$

$$\Delta = k_{mf}^{-1} - k_{mr}^{-1} \tag{18}$$

As indicated in previous studies (Chen and Wu, 1987; Palazzi and Converti, 1997; Park et al., 1981), a characteristic temperature, T_L, exists in the range of practical interest for industrial applications (60° to 80°C), where:

$$k_{mf} = k_{mr} = k_m$$

In this situation, we have $\Delta = 0$ and then:

$$\lambda = \lambda_L = k_m^{-1} \tag{19}$$

$$k''_i = \frac{1}{1 + k_m^{-1}\,G_o} = k''_i\,(G_o) = k''_{i_L} \tag{20}$$

This means that k_i also becomes independent of G at the characteristic temperature, T_L, and varies with the feed concentration, G_o:

$$k_i = k_i\,(G_o) = k_{i_L} \tag{21}$$

Thus, glucose isomerization behaves as a first-order reversible process described by the relationship:

$$v' = k_{i_L}\,(G - G_e) \tag{22}$$

In the extreme situation of a process in which this concentration varies from G_o to G_e, λ will vary from $\lambda_o = \lambda(G_o)$ to $\lambda_e = \lambda(G_e)$, where:

$$\lambda_o = k_{mf}^{-1} \tag{23}$$

$$\lambda_e = k_{mf}^{-1} - \frac{K}{K + 1}\Delta \tag{24}$$

Enzyme Inactivation Kinetics

According to Chen and Wu (1987), the residual activity of an enzyme subject to thermal inactivation varies with the time as:

$$\frac{d\psi}{dt} = -k_d\psi \tag{25}$$

where the first-order inactivation constant in the presence of substrate protection, k_d, is given by:

$$k_d = k'_d\,(1 - \sigma) \tag{26}$$

k'_d being the first-order inactivation constant in the absence of substrate protection, σ the so-called protection factor:

$$\sigma = \frac{n[k_{mr}\,(1 + G/G_e) + k_{mf}\,(K\text{-}G/G_e)]}{k_{mf}k_{mr}/G_e + [k_{mr}\,(1 + G/G_e) + k_{mf}\,(K\text{-}G/G_e)]} \tag{27}$$

and n a factor related to the effectiveness of substrate protection. Chen and Wu (1987) demonstrated that this factor is 0.5 for glucose isomerase inactivation, thus it should be noticed that:

$$\lim_{G \to +\infty} \sigma = n = 0.5$$

which means that the constant of thermal deactivation tends to a half at very high substrate concentration. On the other hand, it is not possible to achieve a value of $\sigma = 1$, which would correspond to the ideal situation of no deactivation.

On closer examination of Eq. (26), it is evident that the inclusion of the protection factor in k_d forces the substrate concentration to be buried in this parameter and thus the inactivation rate is, in general, reduced due to the presence of the substrate.

It is also convenient to split parameter k_d, from Eq. (26), as follows:

$$k_d = k'_d\,(T)k''_d\,(G, G_o, T) \tag{28}$$

where

$$k''_d = \frac{1 + (1 - n)\,\mu\,G_o}{1 + \mu\,G_o} \tag{29}$$

$$\mu = k_{mf}^{-1} - \Delta\left(\frac{K}{K+1} - \frac{G}{G_o}\right) = \mu\,(G, G_o, T) \tag{30}$$

The simultaneous dependence of k_d on G, G_o, and T, described in Eq. (28), was verified in previous studies (Chen and Wu, 1987; Converti and Del Borghi, 1997, 1998). In particular, this parameter increases remarkably with temperature according to the Arrhenius equation and gradually decreases with both G and G_o at a given temperature.

In the particular situation where $T = T_L$, we have:

$$\mu = \mu_L = k_m^{-1} \tag{31}$$

$$k''_d = \frac{1 + (1 - n)\,k_m^{-1}\,G_o}{1 + k_m^{-1}\,G_o} = k''_d\,(G_o) = k''_{d_L} \tag{32}$$

Then, according to Eq. (28), k_d becomes independent of G:

$$k_d = k_d\,(G_o) = k_{d_L} \tag{33}$$

It should be noted that, although k_d is in general a function of G [see Eqs. (26), (27), and (30)], in the particular case of the characteristic temperature, T_L, it becomes independent of it and can vary with the feed concentration, G_o, only. As explained in more detail in what follows, this peculiar dual behavior of the inactivation constant was suggested by the results of a previous study (Palazzi and Converti, 1997) carried out at a temperature (75°C) very close to the characteristic temperature, T_L.

In the extreme case of a process in which glucose concentration varies from G_o to G_e, μ will vary from $\mu_o = \mu\,(G_o)$ to $\mu_e = \mu\,(G_e)$, where:

$$\mu_o = k_{mf}^{-1} + \frac{1}{K+1}\Delta \tag{34}$$

$$\mu_e = k_{mf}^{-1} - \frac{K-1}{K+1}\Delta \tag{35}$$

Simplified Reaction Kinetics

Because industrial processes usually employ immobilized enzymes, the knowledge of the concentration behavior within the catalyst pellet as well as along the reactor is fundamental in process design and optimization. Obtaining these concentration profiles generally requires the integration of a suitable glucose balance together with the instantaneous local balance of the active enzyme, Eq. (25), coupled with Eq. (1). This is due to the fact that, in a generic process, both temperature and substrate concentration in the neighborhood of a given molecule of enzyme can vary with time. Also, Eq. (28) indicates that parameter k_d generally varies with time. In this situation, even the use of numerical methods (Verhoff and Goldstein, 1982) does not make integration of the simultaneous differential equations easy.

The main methods by which to simplify the mathematical problem substantially involve the possibility of decoupling the glucose and active enzyme balances, and by linearizing the isomerization kinetics. This can be accomplished if the following hypotheses are verified:

- Pseudo steady-state conditions in the catalyst pellet.
- Isothermal process.
- Kinetic constant for enzyme inactivation, k_d, practically independent of glucose concentration, G.
- Kinetic constant for the isomerization, k_i, practically independent of glucose concentration, G.

The hypothesis of pseudo-steady-state conditions, which allows one to neglect the accumulation term in the glucose balance within the catalyst pellet, is generally accepted (Houng et al., 1993; Illanes et al., 1996). Because of the great number of temperature-dependent parameters usually present in the model equations available in the literature, assuming isothermal conditions, whenever applicable, would be a relevant simplification in the attempt to come up with an analytical solution to the problem.

In the following, a theoretical discussion will be developed on the validity and significance of the last two assumptions, namely the negligible dependence of both k_d and k_i on glucose concentration at temperatures close to the characteristic temperature, T_L.

Glucose Isomerization at Characteristic Temperature, T_L

If glucose isomerization is carried out at the characteristic temperature, T_L, k_d can depend only on G_o, as indicated in Eq. (33), then it keeps constant during the entire process. Eq. (25), which is now decoupled from the glucose balance,

can be integrated with respect to the time, to obtain the behavior of the enzyme residual activity:

$$\int_1^{\psi} d\psi/\psi = -\int_0^t k_{d_L}\, dt \tag{36}$$

The result can be written in the form:

$$\psi = \exp(-k_{d_L}\, t) \tag{37}$$

As far as the isomerization kinetics are concerned, the specific isomerization rate depends linearly, at the characteristic temperature, T_L, on glucose concentration, according to Eq. (22). Then, in the particular situation considered here, Eq. (1) becomes:

$$v = [k_{i_L} \exp(-k_{d_L}\, t)]\,(G - G_e) \tag{38}$$

where the factor within the square brackets is a rather simple function of G_o and of the catalyst age, *t*. This last equation can be used directly to evaluate the diffusional resistances and to properly design a process carried out exactly at temperature T_L, at which $k_{mf} = k_{mr}$. Moreover, it could also be utilized, as a first approximation, to obtain useful information for carrying out isomerization at a temperature not too different from T_L, which, on the other hand, seems to be very close to that required for the optimization of an isothermal process.

Glucose Isomerization at Constant Temperature Different from T_L

Because the actual rate of glucose isomerization at the characteristic temperature is a linear function of glucose concentration [Eq. (38)], a similar behavior can also reasonably be assumed at temperatures close to T_L.

The validity of this assumption has recently been verified (Palazzi and Converti, 1997) at 75°C, where $k_{mf} \neq k_{mr}$. In fact, the maximum relative error introduced by this linearization was 3.6% at $G_o = 3000$ mol m^{-3} and remarkably decreased with decreasing G_o. Moreover, the dependence of the inactivation rate on glucose concentration, *G*, also proved extremely weak at 75°C and the difference between the maximum and minimum values of $(1 - \sigma)$, calculated at different G_o values, never exceeded 2.0%.

MATERIALS AND METHODS

Commercial Sweetzyme T, supplied by Novo Nordisk (Milan, Italy), is an active granular GI (E.C. 5.3.1.5 D-xylose ketolisomerase) produced from a selected strain of *Streptomyces murinus* and supported on silica. The characteristics of the commercial preparation were: dry specific activity of 350 IGIU g^{-1}, water absorbing power of 1.0 to 1.3 g g^{-1}, and particle density of 1.43 g cm^{-3}. To minimize the intraparticle diffusion resistance within the supporting matrix, the enzyme particles were reduced up to an average diameter of less than 0.3 mm and used in a well-mixed isomerization reactor.

Fructose and glucose were determined using HPLC (Waters ALC-201) with an IR detector. A column (Bio-Rad HPX-87C) was used with bidistilled water as mobile phase at a flow rate of 0.5 mL min^{-1}.

A solution was prepared by adding 450 g of glucose to 1 L of a 0.05 *M* Tris-buffer solution at pH 7.0 containing 20 g $MgSO_4 \cdot 7\ H_2O$ and 20 g Na_2SO_3. The activity tests were performed in 125-mL Erlenmeyer flasks in which 0.6 g of fresh immobilized enzyme was added to 50 mL of the aforementioned solution and shaking the flasks in a water bath at 60°C. Samples were analyzed periodically for glucose and fructose to estimate the starting reaction rate. The residual activity of the enzyme was checked periodically in the same way, after withdrawing, from the well-mixed isomerization reactor, suspension volumes corresponding to catalyst samples of 0.6 g. It was then referred to the starting activity of the fresh enzyme.

Kinetic parameters were estimated through batch runs carried out at pH 7.0 at different temperatures using the same enzyme concentration as that of the activity tests.

The parameters of thermal inactivation were evaluated by means of long-term tests, which started after addition of about 50 g fresh immobilized enzyme and lasted 50 to 90 h. The activity decrease was followed by regular withdrawal of enzyme samples every 10 to 15 h. They were immediately washed (twice) with a fresh buffer solution to exclude any possible influence of reversible inactivation on permanent inactivation, and were submitted to activity tests for estimation of the activity coefficient. Thermal inactivation studies in the presence of substrate protection were carried out in the same well-stirred vessel, varying the equilibrium concentration according to the selected temperature.

EXPERIMENTAL RESULTS

Tests in Absence of Inactivation

The kinetic results shown in Figures 1 and 2, that have been obtained for both forward and reverse reactions through batch isomerization tests at pH 7.0 and $G_o = 2000$ mol m^{-3} using 12 g L^{-1} of enzyme, compare well with those calculated by Chen and Wu (1987) for a less active immobilized GI. In particular, Figure 2 shows that the condition $k_{mf} = k_{mr} = k_m = 854$ mol m^{-3} is verified when $T = T_L = 70.22$°C.

A comparison of the equilibrium constants listed in Table I with those reported in the literature for the native enzyme (Roels, 1983) shows that immobilization surprisingly favors the equilibrium for $T > 70$°C, whereas, below this temperature, the native enzyme allows a more complete conversion.

As far as the effects of initial substrate concentration on immobilized glucose isomerase activity is concerned, the results presented in a previous work (Converti and Del Borghi, 1997) showed, as expected, that v'_m is not influenced, being a parameter consisting of only a combination of kinetic constants. On the contrary, K_m, which is a combination of both kinetic parameters and G_o, shows a nearly linear increase within the whole tested range of G_o.

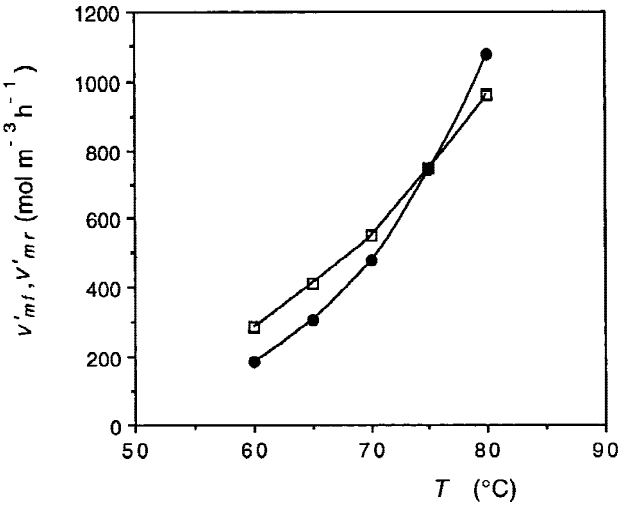

Figure 1. Maximum velocities of (❐) forward and (●) reverse reactions of glucose isomerization to fructose at different temperatures.

Tests in Presence of Inactivation

The residual activity of the enzyme has been determined, within the same temperature range, at time intervals much longer than those selected for enzyme activity tests. The enzyme inactivation tests in the absence of substrate protection were carried out in a buffer solution at pH 7.0, whereas four different solutions with variable total sugar equilibrium concentrations were used for tests in the presence of substrate protection. The results in terms of apparent first-order inactivation constant, k'_d, are shown in Figure 3. As expected, the apparent first-order inactivation constant increases remarkably with increasing temperature.

Also, the occurrence of a substrate protection phenomenon was confirmed in the same work (Converti and Del Borghi, 1997) by the increase in the experimental values of σ with G_o. The satisfactory agreement between these values, calculated via the apparent first-order inactivation constant, with the ones estimated in this work utilizing the kinetic parameters of Figures 1 and 2 provides a confirmation of the validity not only of assuming first-order kinetics for thermal inactivation of the enzyme but also of the value $n = 0.5$ calculated by Chen and Wu (1987).

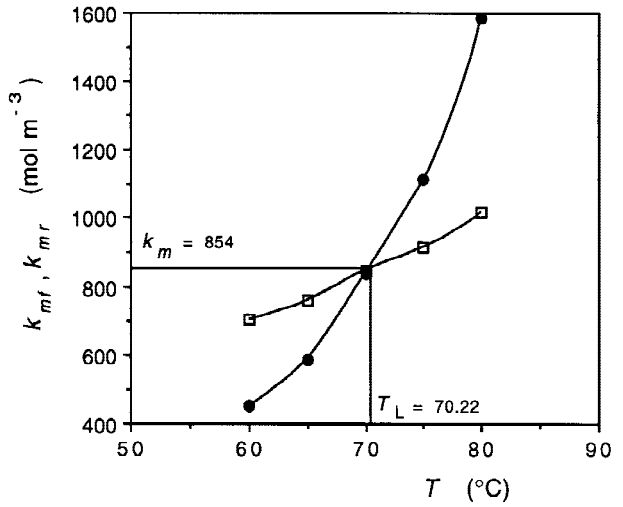

Figure 2. Michaelis constants of (❐) forward and (●) reverse reactions of glucose isomerization to fructose at different temperatures.

Estimation of Kinetic Parameters

Table I summarizes the values of the main parameters to which the following discussion on the linearization of isomerization kinetics will refer. In particular, $\underline{\lambda}$ and $\underline{\mu}$ have been defined as the arithmetic means of λ and μ values in the feed and at the thermodynamic equilibrium:

$$\underline{\lambda} = \frac{\lambda_o + \lambda_e}{2} \tag{39}$$

$$\underline{\mu} = \frac{\mu_o + \mu_e}{2} \tag{40}$$

LINEARIZATION OF KINETICS

The validity of assuming linear kinetics is verified here within the whole ranges of temperature (60° to 80°C) and concentration (500 to 3000 mol m^{-3}) of practical interest. Because the errors introduced by the linearization increase as T deviates from T_L, the extreme temperatures of this interval (60° to 80°C) correspond to the less favorable situations and have thus been selected for this purpose.

Dependence of Decay Kinetic Constant on Substrate Concentration

As already noted, if $T = T_L$, $k''_d = k''_{d_L}$ becomes independent of G. Moreover, for $T < T_L$, Δ becomes negative, so k''_d increases with G. Under these conditions, we have:

$$k''_{d_e} \leq k''_d \leq k''_{d_o} \tag{41}$$

where:

$$k''_{d_e} = k''_d(\mu_e) = \frac{1 + (1-n)\,\mu_e\,G_o}{1 + \mu_e\,G_o} \tag{42}$$

$$k''_{d_o} = k''_d(\mu_o) = \frac{1 + (1-n)\,\mu_o\,G_o}{1 + \mu_o\,G_o} \tag{43}$$

The opposite situation takes place when $T > T_L$, so:

$$k''_{d_o} \leq k''_d \leq k''_{d_e} \tag{44}$$

First, let the range of function k''_d (G) be defined as:

$$\omega_d = |k''_{d_o} - k''_{d_e}| = \omega_d\,(G_o, T) \tag{45}$$

The values of k''_{d_o} and k''_{d_e}, calculated at 60° and 80°C when G_o varies within the whole experimental domain, are reported in Table II, together with the respective range, ω_d. As demonstrated in Appendix 1, the maximum value of the range ($\omega_d = 0.0406$) is reached when $G_o = 833$ mol m^{-3}

Table I. Values of some parameters calculated using the experimental data of Figures 1 and 2, through the equations listed in the last column.

T (°C)	60	65	70	70.22[b]	75	80	Eq.
K	0.982	1.028	1.140	1.145	1.223	1.390	6
k'_i (h^{-1})	0.817	1.064	1.209	1.221	1.481	1.623	15
$\Delta \cdot 10^3$ ($m^3\ mol^{-1}$)	−0.792	−0.388	−0.013	0	0.191	0.353	18
$\lambda_o \cdot 10^3$ ($m^3\ mol^{-1}$)[a]	1.420	1.312	1.175	1.171	1.091	0.984	23
$\lambda_e \cdot 10^3$ ($m^3\ mol^{-1}$)	1.812	1.509	1.182	1.171	0.986	0.779	24
$\underline{\lambda} \cdot 10^3$ ($m^3\ mol^{-1}$)	1.616	1.410	1.178	1.171	1.038	0.881	39
$\mu_o \cdot 10^3$ ($m^3\ mol^{-1}$)	1.020	1.121	1.169	1.171	1.177	1.132	34
$\mu_e \cdot 10^3$ ($m^3\ mol^{-1}$)	1.413	1.317	1.177	1.171	1.072	0.926	35
$\underline{\mu} \cdot 10^3$ ($m^3\ mol^{-1}$)	1.216	1.219	1.173	1.171	1.124	1.029	40

[a]$\lambda_o = k_{mf}^{-1}$. [b]Values estimated by linear interpolation.

and $T = 60$°C and corresponds only to 5.27% of k''_{d_o}. Moreover, it is important to note from the results of Table II that not only the deactivation constant, k_d (Converti and Del Borghi, 1997, 1998), but also the parameters k''_{d_o} and k''_{d_e} decrease with an increase in G_o, due to substrate protection of the enzyme.

Because the range of k''_d is very narrow, it seems reasonable to approximate the expression of the kinetic constant for enzyme deactivation, k''_d (G, G_o, T), between G_e and G_o, with a suitable mean value, k''_{d_j} (G_o, T), which is independent of G.

As shown in Table III, various expressions of this kind have been considered. The first three formulas have been derived, as explained in Appendix 1, with the aim of minimizing the errors involved in the approximation, either with respect to k''_d or its mean value, $\underline{k}''_d$, within the interval (G_e, G_o). For this purpose, the use of $\underline{k}''_d$ seems to be more appropriate for comparison, because glucose concentration in contact with the enzyme in industrial processes will generally vary with time.

The values of the kinetic parameters of glucose isomerase inactivation calculated by these three approximating functions are compared in Table IV with those estimated using the fourth expression of Table III:

$$k''_{d_4} = \frac{1 + (1 - n)\,\underline{\mu}\,G_o}{1 + \underline{\mu}\,G_o} \tag{46}$$

Eq. (46) seems to be the best tool for approximating the theoretical behavior of k''_d, because it allows for minimization of the aforementioned errors while retaining the simple algebraic form of Eq. (29). A still simpler version of this equation is Eq. (32), where $k''_d = k''_{d_L}$ depends on G_o only. As pointed out in Figure 4, just because k''_{d_L} is independent of T, the errors involved in this approximation (1.8% to 2.2% at 80°C) are obviously larger than those implied by use of Eq. (46) (0.02% to 0.05%).

Dependence of Isomerization Kinetic Constant on Substrate Concentration

The dependence of k''_i on G, described by Eq. (16), is of the same type as that observed for k''_d. In particular, if $T = T_L$, $k''_i = k''_{i_L}$ becomes independent of G and, if $T < T_L$, we have:

$$k''_{i_e} \leqslant k''_i \leqslant k''_{i_o} \tag{47}$$

where:

$$k''_{i_e} = k''_i\,(\lambda_e) = \frac{1}{1 + \lambda_e\,G_o} \tag{48}$$

$$k''_{i_o} = k''_i\,(\lambda_o) = \frac{1}{1 + \lambda_o\,G_o} \tag{49}$$

whereas the opposite takes place when $T > T_L$:

$$k''_{i_o} \leqslant k''_i \leqslant k''_{i_e} \tag{50}$$

Based on the analogy in the previous section, let the range of the function k''_i (G) be defined as:

$$\omega_i = |k''_{i_o} - k''_{i_e}| = \omega_i\,(G_o, T) \tag{51}$$

From the values of k''_{i_o}, k''_{i_e}, and ω_i, calculated at 60° and 80°C, when G_o varies within the whole experimental domain (Table II), it should be noted that the range ω_i, like ω_d, is rather narrow, both in an absolute and relative sense.

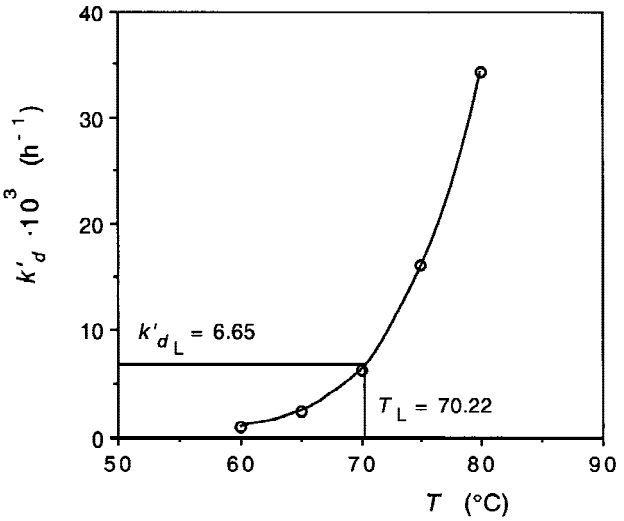

Figure 3. Dependence of the decay constant on the temperature in the absence of substrate protection.

Table II. Influence of temperature and glucose concentration in the feed on the main kinetic parameters of enzyme inactivation and glucose isomerization.

	Enzyme inactivation			Glucose isomerization		
G_o (mol m^{-3})	k''_{d_o}	k''_{d_e}	ω_d	k''_{i_o}	k''_{i_e}	ω_i
T = 60°C						
500	0.8311	0.7930	0.0381	0.5848	0.5247	0.0601
1000	0.7475	0.7072	0.0403	0.4132	0.3556	0.0576
2000	0.6645	0.6307	0.0338	0.2604	0.2163	0.0441
3000	0.6232	0.5954	0.0278	0.1901	0.1554	0.0347
T = 80°C						
500	0.8193	0.8418	0.0225	0.6702	0.7197	0.0495
1000	0.7345	0.7596	0.0251	0.5040	0.5621	0.0581
2000	0.6532	0.6753	0.0221	0.3369	0.3909	0.0540
3000	0.6137	0.6323	0.0186	0.2530	0.2997	0.0467

Because k''_i is not very sensitive to variations of G, the isomerization rate, v', can be considered approximately as a straight line, by virtue of Eq. (13). This is confirmed in Figure 5, where the behavior of the function v' (G) is illustrated for G_o = 3000 mol m^{-3} and T = 60, 70.22, and 80°C, respectively.

Therefore, we can attempt to linearize Eq. (13) by using a suitable mean value of the function k''_i, between G_e and G_o, which depends only on G_o and T. As illustrated in Table V, three different expressions of k''_i have been considered with the aim of minimizing the errors on v' and $\underline{v}'$ involved in the approximation, $\underline{v}'$ being the mean value of v' within the interval (G_e, G_o). Comparing in Table IV the values calculated for the parameter k''_i with these approximating functions, one can observe that the proposed expressions can be considered practically equivalent from a numerical point of view. Nevertheless, the last formula derived in Appendix 2:

$$k''_{i_3} = \frac{1}{1 + \lambda' G_o} \tag{52}$$

where:

$$\lambda' = \frac{2\,\lambda_o + \lambda_e}{3} \tag{53}$$

seems preferable for practical applications, because it has the same algebraic form of Eq. (16) and practically gives the mean value of the specific isomerization rate, v', within the whole range of glucose concentration (Fig. 6 and Appendix 2).

In conclusion, contrary to the approximation of k''_d with k''_{d_L}, the corresponding approximation of k''_i with k''_{i_L} [Eq. (20)] implies unacceptable errors.

Linearized Kinetics

In the previous sections it has been demonstrated that, for any temperature $T \neq T_L$ ranging from 60° to 80°C, the actual rate of glucose isomerization to fructose, v, can reasonably be approximated by the equation:

$$v = [k_{i_j} \exp(-k_{d_j} t)]\,(G - G_e) \tag{54}$$

The linearized expressions of k_{i_j} and k_{d_j}, which best meet

Table III. Alternative functions independent of G approximating the parameter k''_d within the interval (G_e, G_o).

Proposed forms of k''_d	Description	Characteristics
$k''_{d_1} = (k''_{d_o} + k''_{d_e})/2$	Arithmetic mean of k''_{d_o} and k''_{d_e}	Minimizes the maximum absolute error on k_d ($\varepsilon_{d,\max}$): min $\{\varepsilon_{d,\max}\}$ = 0.0203
$k''_{d_2} = \dfrac{2\,k''_{d_o}\,k''_{d_e}}{k''_{d_o} + k''_{d_e}}$	Ratio between the square of the geometric mean and the arithmetic mean of k''_{d_o} and k''_{d_e}	Minimizes the maximum relative error on k_d ($\varepsilon_{r_{d,\max}}$): min $\{\varepsilon_{r_{d,\max}}\}$ = 0.0278
$k''_{d_3} = \underline{k}''_d = \dfrac{1}{G_o - G_e}\displaystyle\int_{G_e}^{G_o} k''_d\,\mathrm{d}G$	Mean value of k''_d in the interval (G_e, G_o)	Annuls $\underline{\varepsilon}_d$ and $\underline{\varepsilon}_{r_d}$
$k''_{d_4} = \dfrac{1 + (1-n)\,\underline{\mu}\,G_o}{1 + \underline{\mu}\,G_o}$	Value of k''_d calculated for μ equal to the arithmetic mean of μ_o and μ_e	Expression with the same form as that of Eq. (29)
$k''_{d_L} = \dfrac{1 + (1-n)\,k_m^{-1}\,G_o}{1 + k_m^{-1}\,G_o}$	Theoretical expression of k''_d for $T = T_L$	Expression independent of T

Table IV. Influence of temperature and glucose concentration in the feed on the kinetic parameters of enzyme inactivation (k''_d) and glucose isomerization (k''_i) described by different linearized functions.

	Enzyme inactivation					Glucose isomerization		
G_o (mol m^{-3})	k''_{d_1}	k''_{d_2}	k''_{d_3}	k''_{d_4}	k''_{d_L}	k''_{i_1}	k''_{i_2}	k''_{i_3}
T = 60°C								
500	0.8120	0.8117	0.8121	0.8109	0.8154	0.5531	0.5629	0.5632
1000	0.7273	0.7268	0.7268	0.7256	0.7303	0.3823	0.3922	0.3920
2000	0.6476	0.6472	0.6467	0.6456	0.6496	0.2363	0.2441	0.2438
3000	0.6093	0.6090	0.6084	0.6075	0.6108	0.1710	0.1771	0.1769
T = 80°C								
500	0.8305	0.8304	0.8303	0.8301	0.8154	0.6942	0.6858	0.6859
1000	0.7470	0.7469	0.7466	0.7464	0.7303	0.5316	0.5217	0.5219
2000	0.6642	0.6641	0.6638	0.6635	0.6496	0.3621	0.3533	0.3531
3000	0.6230	0.6229	0.6226	0.6223	0.6108	0.2745	0.2670	0.2668

the different requirements of easiness, error minimizations, etc., are Eqs. (46) and (52), which contain the parameters defined in the Eqs. (23), (24), (34), (35), (39), (40), and (53). The more T tends to T_L, the more the values of k_{i_j} and k_{d_j} approach those of k_{i_L} and k_{d_L}, respectively, which means that Eq. (54) practically concides with Eq. (38).

CONCLUSIONS

To provide data for optimization of an immobilized glucose isomerase reactor, batch tests of glucose isomerization to fructose have been carried out at different temperatures, both in the presence and absence of thermal inactivation of the enzyme as well as under different conditions of substrate saturation. A pseudo-linear model [Eq. (54)] has been used successfully to describe glucose isomerization kinetics. The model, a very satisfactory approximation of the Briggs–Haldane approach, substantially simplifies the well-known problem of optimizing an industrial fixed-bed column for high-fructose corn syrup (HFCS) production.

Our further investigation in this field will deal with the exploitation of this simplification for practical purposes, including:

- The evaluation of both inner and outer diffusional resistances.
- The design of a tubular catalytic reactor for the continuous production of HFCS with constant composition.
- The process optimization from both technical and economic points of view.

APPENDIX 1: APPROXIMATING k''_d (G, G_o, T) WITH k''_{d_j} (G_o, T)

Maximum Range of k''_d (G, G_o, T) within the Interval (G_e, G_o)

The expression of the maximum range of k''_d (G, G_o, T) within the interval (G_e, G_o) can be obtained from Eqs. (29) and (45):

$$\omega_{d,\max} = \max_{(G_o,\,T)} \left\{ \frac{n\,G_o\,|\mu_o - \mu_e|}{(1+\mu_o\,G_o)\,(1+\mu_e\,G_o)} \right\} \qquad (55)$$

where μ_o and μ_e are given by Eqs. (34) and (35).

Considering now the dependence of ω_d on G_o, it is easy to show that, at each temperature, the range reaches its maximum value when:

$$G_o = \frac{1}{\sqrt{\mu_o\,\mu_e}} \qquad (56)$$

Substituting the values of μ_o and μ_e listed in Table I, this equation allows calculation of G_o values of 833 and 977 mol m^{-3} and ω_d values of 0.0406 and 0.0251 at 60° and 80°C, respectively. Then, the highest value of the range in the experimental domain, reached when G_o = 833 mol m^{-3} and T = 60°C, is $\omega_{d,\max}$ = 0.0406.

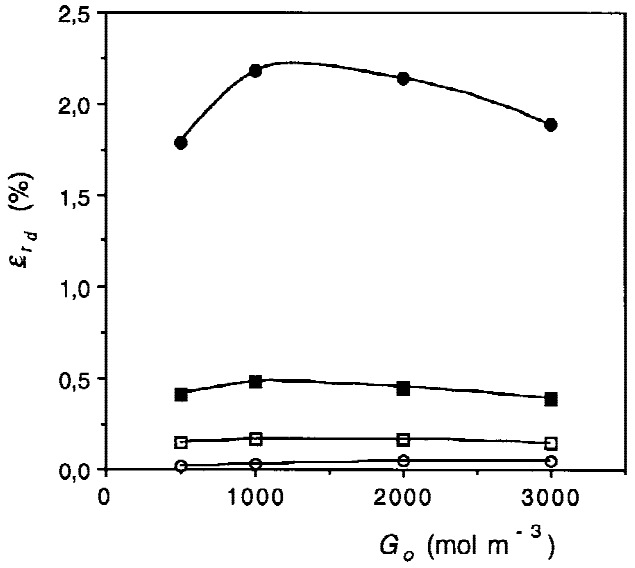

Figure 4. Variability of the relative errors on the parameter k''_d, resulting from the approximation of k''_d with k''_{d_j} [Eq. (71)] within the tested ranges of temperature and glucose concentration in the feed. T = 60°C: (■) k''_{d_L}; (❒) k''_{d_4}. T = 80°C: (●) k''_{d_L}; (○) k''_{d_4}.

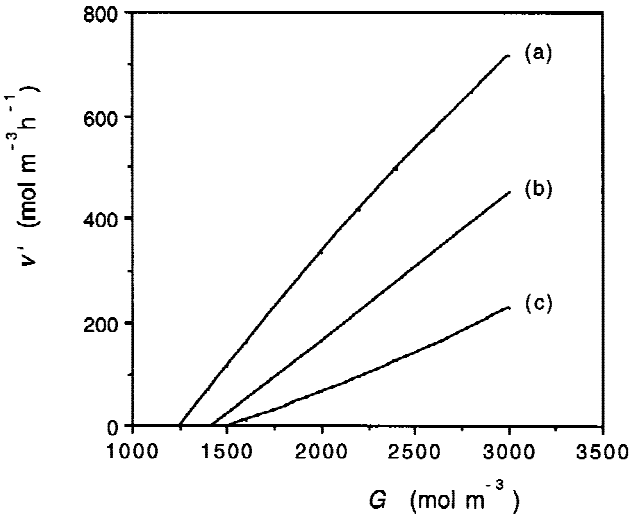

Figure 5. Dependence of the specific isomerization rate of the fresh catalyst on glucose concentration. (a) $T = 80$°C; (b) $T = T_L = 70.22$°C; (c) $T = 60$°C.

Approximation Minimizing the Maximum Absolute Error on k''_d

The maximum absolute error on k''_d is defined as:

$$\varepsilon_{d,\max} = \max\{\varepsilon_d\} = \max_{(G, G_o, T)} \{|k''_{d_j} - k''_d|\} \tag{57}$$

where the subscripts j = 1,2,3,4 refer to four different linearized forms defined in Table III.

Considering the inequalities (41) and (44), we have:

$$\varepsilon_{d,\max} = \max_{(G_o, T)} \{|k''_{d_j} - k''_{d_o}|, |k''_{d_j} - k''_{d_e}|\} \tag{58}$$

By putting:

$$|k''_{d_j} - k''_{d_o}| = |k''_{d_j} - k''_{d_e}| \tag{59}$$

the value of k''_{d_j}, which minimizes $\varepsilon_{d,\max}$, is obtained:

$$k''_{d_j} = \frac{k''_{d_o} + k''_{d_e}}{2} \tag{60}$$

from which the maximum absolute error on k_d is:

$$\varepsilon_{d,\max} = \max_{(G_o, T)} \{\omega_d/2\} \tag{61}$$

So, repeating the same reasoning followed in the previous section, one can easily obtain the highest value of ε_d within the experimental domain:

$$\varepsilon_{d,\max} = \omega_{d,\max}/2 = 0.0203 \tag{62}$$

Approximation Minimizing the Maximum Relative Error on k''_d

The maximum relative error on k''_d is defined as:

$$\varepsilon_{r_{d,\max}} = \max\{\varepsilon_{r_d}\} = \max_{(G, G_o, T)} \{|1 - k''_{d_j}/k''_d|\} \tag{63}$$

Considering the inequalities (41) and (44), we have:

$$\varepsilon_{r_{d,\max}} = \max_{(G_o, T)} \{|1 - k''_{d_j}/k''_{d_o}|, |1 - k''_{d_j}/k''_{d_e}|\} \tag{64}$$

By putting:

$$|1 - k''_{d_j}/k''_{d_o}| = |1 - k''_{d_j}/k''_{d_e}| \tag{65}$$

the value of k''_{d_j}, which minimizes $\varepsilon_{r_{d,\max}}$, is obtained:

$$k''_{d_j} = \frac{2\, k''_{d_o}\, k''_{d_e}}{k''_{d_o} + k''_{d_e}} \tag{66}$$

from which the maximum relative error on k_d results to be:

$$\varepsilon_{r_{d,\max}} = \max_{(G_o, T)} \left\{ \frac{n\, G_o\, |\mu_o - \mu_e|}{2 + (2-n)(\mu_o + \mu_e) G_o + 2\,(1-n)\,\mu_o\,\mu_e\, G_o^{\,2}} \right\} \tag{67}$$

Table V. Alternative functions independent of G approximating the parameter k''_i within the interval (G_e, G_o).

Proposed forms of k''_i	Description	Characteristics
$k''_{i_1} = \dfrac{2\, k''_{i_o}\, k''_{i_e}}{k''_{i_o} + k''_{i_e}} = \dfrac{1}{1 + \underline{\lambda}\, G_o}$	Ratio between the square of the geometric mean and the arithmetic mean of k''_{i_o} and k''_{i_e}, or value of k''_i calculated for λ equal to the arithmetic mean of λ_o and λ_e	Minimizes the maximum relative error on k_i ($\varepsilon_{r_{i,\max}}$): min $\{\varepsilon_{r_{i,\max}}\} = 0.1005$; expression with the same form as that of Eq. (16)
$k''_{i_2} = \dfrac{2}{(\lambda_o - \lambda_e)\, G_o} \left[1 - \dfrac{\ln(1+\varphi)}{\varphi} \right]$	Value of k''_i, independent of G, for which the linearized kinetics give the theoretical mean value ($\underline{v}'$) of v' in the interval (G_e, G_o)	Annuls $\underline{\varepsilon}_i$ and $\underline{\varepsilon}_{r_i}$; φ defined in Eq. (80)
$k''_{i_3} = \dfrac{1}{1 + \lambda'\, G_o}$	Value of k''_i calculated for $\lambda' = (2\,\lambda_o + \lambda_e)/3$	Expression with the same form as that of Eq. (16)

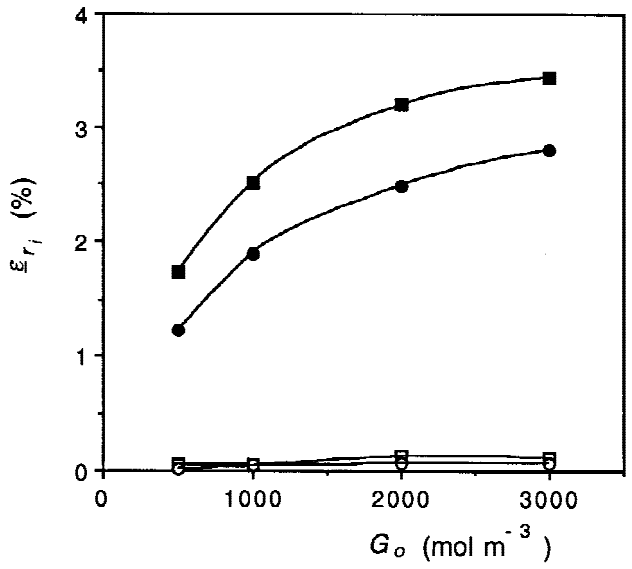

Figure 6. Variability of the relative errors on the parameter k''_{i_2} resulting from the approximation of k''_i with k''_{i_j} [Eq. (86)] within the tested ranges of temperature and glucose concentration in the feed. $T = 60$°C: (■) k''_{i_1}; (❒) k''_{i_3}. $T = 80$°C: (●) k''_{i_1}; (○) k''_{i_3}.

At each temperature, the relative error $\varepsilon_{r_{d,\max}}$ becomes maximum when:

$$G_o = \frac{1}{\sqrt{(1-n)\,\mu_o\,\mu_e}} \tag{68}$$

Substituting $n = 0.5$ and the values of μ_o and μ_e listed in Table I, this equation allows calculation of G_o values of 1079 and 1381 mol m^{-3}, and ε_{r_d} values of 0.0278 and 0.0172 at 60° and 80°C, respectively. Then, the highest value of the relative error in the experimental domain, reached when $G_o = 1079$ mol m^{-3} and $T = 60$°C, is $\varepsilon_{r_{d,\max}} = 0.0278$.

Mean Value of k'_d (G, G_o, T) in the Interval (G_e, G_o)

Let $\underline{k}''_d$ (G_o, T) be the mean value of k''_d (G, G_o, T) in the interval (G_e, G_o). We have:

$$\underline{k}''_d = \frac{1}{G_o - G_e}\int_{G_e}^{G_o} k''_d \mathrm{d}G \tag{69}$$

Considering the Eqs. (29) and (30), we obtain:

$$\underline{k}''_d = 1 + \frac{n}{(\mu_o - \mu_e)\,G_o}\ln\left[1 + \frac{(\mu_o - \mu_e)\,G_o}{1 + \mu_e\,G_o}\right] \tag{70}$$

The mean relative error on $\underline{k}''_d$, due to the approximation of k''_d with a particular form of k''_{d_j}, is:

$$\underline{\varepsilon}_{r_d} = |1 - k''_{d_j}/\underline{k}''_d| \tag{71}$$

Figure 4 shows the values of this error for the two last forms of k''_{d_j} reported in Table III, namely k''_{d_4} and k''_{d_L}.

APPENDIX 2: APPROXIMATING k''_i (G, G_o, T) WITH k''_{i_j} (G_o, T)

Approximation Minimizing the Maximum Relative Error on k''_i

The maximum relative error on k''_i, defined as:

$$\varepsilon_{r_{i,\max}} = \max\{\varepsilon_{r_i}\} = \max_{(G,\,G_o,\,T)}\{|1 - k''_{i_j}/k''_i|\} \tag{72}$$

coincides, by virtue of linearization of Eq. (13) using a mean value of k''_i, with the maximum relative error on v'.

Considering the inequalities (47) and (50), we have:

$$\varepsilon_{r_{i,\max}} = \max_{(G_o,\,T)}\{|1 - k''_{i_j}/k''_{i_o}|, |1 - k''_{i_j}/k''_{i_e}|\} \tag{73}$$

By putting:

$$|1 - k''_{i_j}/k''_{i_o}| = |1 - k''_{i_j}/k''_{i_e}| \tag{74}$$

the value of k''_{i_j}, which minimizes $\varepsilon_{r_{i,\max}}$, is obtained:

$$k''_{i_j} = \frac{2\,k''_{i_o}\,k''_{i_e}}{k''_{i_o} + k''_{i_e}} \tag{75}$$

or, alternatively:

$$k''_{i_j} = \frac{1}{1 + \underline{\lambda}\,G_o} \tag{76}$$

Therefore, the maximum relative error on k_i can be calculated:

$$\varepsilon_{r_{i,\max}} = \max_{(G_o,\,T)}\left\{\frac{|\lambda_o - \lambda_e|\,G_o}{2 + (\lambda_o + \lambda_e)G_o}\right\} \tag{77}$$

Because at each temperature the relative error $\varepsilon_{r_{i,\max}}$ increases with G_o, this last equation allows calculation at $G_o = 3000$ mol m^{-3}, of ε_{r_i} values of 0.1005 and 0.0803 at 60° and 80°C, respectively. Then, the highest value of the relative error within the experimental domain, reached when $G_o = 3000$ mol m^{-3} and $T = 60$°C, is $\varepsilon_{r_{i,\max}} = 0.1005$.

Mean Value of v' in the Interval (G_e, G_o)

Let $\underline{v}'$ (G_o, T) be the mean value of v' in the interval (G_e, G_o). We have:

$$\underline{v}' = \frac{1}{G_o - G_e}\int_{G_e}^{G_o} v'\mathrm{d}G \tag{78}$$

Considering Eqs. (13)–(17), we obtain:

$$\underline{v}' = \frac{k'_i}{\Delta}\left[1 - \frac{\ln(1+\varphi)}{\varphi}\right] \tag{79}$$

where:

$$\varphi = \frac{(\lambda_o - \lambda_e)\,G_o}{1 + \lambda_e\,G_o} \tag{80}$$

On the other hand, if a linearized form is assumed for the

specific isomerization rate, with k_{i_j} independent of G, the mean value of v' can be calculated by:

$$\underline{v}' = \underline{v}'_j = \frac{k'_i \, k''_{i_j} \, (G_o - G_e)}{2} \tag{81}$$

By equating the right-hand sides of Eqs. (79) and (81), it is then possible to obtain the particular value of k''_{i_j} (which is independent of G and is indicated in the following as k''_{i_2}) for which the linearized form of v' gives exactly the mean value of v' in the interval (G_e, G_o):

$$k''_{i_2} = \frac{2}{(\lambda_o - \lambda_e) \, G_o} \left[1 - \frac{\ln (1 + \varphi)}{\varphi} \right] \tag{82}$$

Because $\varphi <<< 1$, the McLaurin expansion series of $\ln(1 + \varphi)$:

$$\ln (1 + \varphi) \cong \varphi - \frac{\varphi^2}{2} + \frac{\varphi^3}{3} \tag{83}$$

allows to obtain a simplified form of Eq. (82):

$$k''_{i_2} \cong k''_{i_3} = \frac{1}{1 + \lambda' G_o} \tag{84}$$

where:

$$\lambda' = \frac{\lambda_e + 2 \, \lambda_o}{3} \tag{85}$$

As indicated in Table IV, Eqs. (82) and (83) can be considered equivalent, for practical purposes. The relative error on k''_{i_2}, due to the approximation of k''_i with a particular form of k''_{i_j}, is:

$$\underline{\varepsilon}_{r_i} = |1 - k''_{i_j} / k''_{i_2}| \tag{86}$$

and coincides with the relative error on $\underline{v}'$. Figure 6 shows the values of this error for the alternative forms of k''_{i_j} considered in Table V, namely $k''_{i_j} = k''_{i_1}$ and $k''_{i_j} = k''_{i_3}$.

NOMENCLATURE

E	enzyme
EF	intermediate complex of the reverse reaction
EG	intermediate complex of the forward reaction
F	fructose or product
G	glucose or substrate
GI	glucose isomerase
X	intermediate

Symbols

E	active enzyme concentration (M L^{-3})
G	glucose concentration (M L^{-3})
K	equilibrium constant (—)
k_{+1}	rate constant of EG formation from G and E (L^3 T^{-1} M^{-1})
k_{-1}	rate constant of EF consumption to form G and E (T^{-1})
k_{+2}	rate constant of EG consumption to form F and E (T^{-1})
k_{-2}	rate constant of EF formation from F and E (L^3 T^{-1} M^{-1})
k_d	actual decay constant (T^{-1})
k'_d	decay constant in the absence of substrate protection (T^{-1})
k''_d	parameter defined in Eq. (29) (—)
$\underline{k''_d}$	mean value of k''_d (—)
k_i	kinetic constant of isomerization (T^{-1})
k'_i	parameter defined in Eq. (15) (T^{-1})
k''_i	parameter defined in Eq. (16) (—)
$\underline{k''_i}$	mean value of k''_i (—)
K_m	kinetic parameter defined in Eq. (8) (M L^{-3})
k_m	Michaelis constant for the forward and reverse reactions at the temperature T_L (M L^{-3})
k_{mf}	Michaelis constant of the forward reaction (M L^{-3})
k_{mr}	Michaelis constant of the reverse reaction (M L^{-3})
n	factor related with the effectiveness of substrate protection (—)
t	time (T)
T	temperature (°C)
T_L	characteristic temperature (°C)
v	actual specific isomerization rate (M L^{-3} T^{-1})
v'	specific isomerization rate when all the biocatalyst is active (M L^{-3} T^{-1})
$\underline{v}'$	mean value of v' (M L^{-3} T^{-1})
v'_m	kinetic parameter defined in Eq. (7) (M L^{-3} T^{-1})
v'_{mf}	maximum velocity of the forward reaction (M L^{-3} T^{-1})
v'_{mr}	maximum velocity of the reverse reaction (M L^{-3} T^{-1})

Greek symbols

Δ	kinetic parameter defined in Eq. (18) (L^3 M^{-1})
ε	absolute error (—)
$\underline{\varepsilon}$	mean absolute error (—)
ε_r	relative error (—)
$\underline{\varepsilon_r}$	mean relative error (—)
φ	parameter defined in Eq. (80) (—)
λ	parameter defined in Eq. (17) (L^3 M^{-1})
$\underline{\lambda}$	mean value of λ (L^3 M^{-1})
λ'	parameter defined in Eq. (53) (L^3 M^{-1})
μ	parameter defined in Eq. (30) (L^3 M^{-1})
$\underline{\mu}$	mean value of μ (L^3 M^{-1})
σ	protection factor (—)
ψ	fractional activity of the enzyme (—)
ω_d	range of the parameter k''_d within the interval (G_e, G_o) (—)
ω_i	range of the parameter k''_i within the interval (G_e, G_o) (—)

Subscripts

d	value referred to enzyme inactivation kinetics
e	value at the termodynamic equilibrium
i	value referred to glucose isomerization kinetics
j = 1,2,3,4	approximated linearized expressions independent of G
L	values referred to linear kinetics, theoretically holding at temperature T_L
max	maximum value
min	minimum value
o	starting value or value in the feed
t	total value

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