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Effect of modulation of enzyme inactivation on temperature optimization for reactor operation with chitin-immobilized lactase

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Abstract

Temperature is a critical variable to be optimized in any enzymatic process, producing opposite effects on enzyme activity and inactivation rate. Temperature functions for all kinetic and inactivation parameters were validated for chitin-immobilized yeast lactase (CIL). Enzyme inactivation was described by a two-stage series mechanism. The effect of galactose and lactose on inactivation was determined in terms of modulation factors that were positive for galactose and negative for lactose. Modulation factors were mild functions of temperature in the first stage and strong functions in the second stage of enzyme inactivation, where galactose positive modulation factors increase while lactose negative modulation factors decrease with temperature. Temperature-explicit functions for kinetic and inactivation parameters were incorporated into a scheme to optimize temperature in the simulation of a continuous packed-bed reactor operation with chitin-immobilized lactase, based on an annual cost objective function. Optimum temperature was 20°C at enzyme replacement of 25% residual activity, and increased only slightly at higher replacement frequencies. The effect of modulation factors on reactor design and temperature optimization is presented and discussed. Software for temperature optimization that allows the introduction of variations in all parameters and operational criteria to perform sensitivity analysis was developed.

Keywords: Immobilized lactase; Enzyme inactivation; Modulation factors; Temperature optimization; Enzyme reactors

1. Introduction

Temperature is the most relevant variable to be optimized for enzyme reactor operation. In fact, enzymes are more active at higher temperature but also more labile, so optimum will arise from a balance between these two opposite effects.

Optimization requires temperature-dependent expressions for kinetic and inactivation parameters. Well-known temperature functions have been validated for kinetic parameters of immobilized enzymes, based on Arrhenius type or thermodynamic correlations [1,2]. Thermal inactivation has been frequently described as a simple one-stage first-order mechanism [3–5], but also by higher order [6] and more elaborated series and parallel mechanisms [7–10]. Inactivation rate constants in those models can be Arrhenius-type functions of temperature [11]. However, information on inactivation kinetics has been gathered under non-reactive conditions that hardly predicts the actual behavior within a reactor, where substrates and products can be modulators of

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enzyme stability [12]. These effects are adequately described by modulation factors [13–15]. However, temperature-explicit functions have not been proposed for modulation factors, although the thermal dependence of glucose modulation on immobilized glucose isomerase stability has been reported [16]. Temperature optimization of enzyme reactors has been previously addressed [1,17–19]. However, in only a few cases, the modulation by substrate and products has been considered for reactor design [15,16,20,21] and never within a scheme of temperature optimization.

Results are presented on the determination of temperature-explicit functions for all kinetic and inactivation parameters of chitin-immobilized yeast lactase (CIL), considering the modulation by lactose and galactose, which are the molecules that interact with the enzyme during catalysis. This information is incorporated into a model for continuous packed-bed reactor operation with CIL from which temperature optimum is obtained in terms of a cost objective function.

2. Experimental

2.1. Materials

Lactase from *Kluyveromyces marxianus* NRLL Y-1009 was produced and immobilized on activated chitin as previously reported [15,22], having a specific activity of 350 ± 20 IU/g. Other reagents were of analytical grade and supplied by Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

2.2. Assays

Lactase activity was determined by measuring initial rates of lactose hydrolysis. Hydrolyzed lactose was determined from the glucose produced and assayed enzymatically with kit 510-A from Sigma. Protein was determined according to Bradford [23]. One international unit of lactase (IU) was defined as the amount of enzyme that hydrolyzes 1 μmol of lactose per minute at 40°C in 200 g/l of lactose solution in 0.1 M phosphate buffer, pH 6.6.

2.3. Determination of temperature dependency of kinetic and inactivation parameters

Kinetic parameters were determined in the range of 10–40°C by linear regression of initial rate data on Lineweaver–Burke plots. Diffusional restrictions were negligible [15] so that kinetic parameters can be considered inherent.

Thermal inactivation of CIL was well described by a two-stage series mechanism [24] and transition rate constants were experimentally determined, as previously described [15], for the free enzyme and enzyme–lactose and enzyme–galactose complexes in the range of 20–40°C. As previously established [15], glucose had no effect on thermal inactivation, which is consistent with the hypothesis that modulation is produced by species (i.e. substrates, inhibitors) that interact with the enzyme during catalysis [12]. The same holds for higher oligosaccharides, whose effect on CIL inactivation were not tested; besides, they are produced in negligible amounts under usual reaction conditions. Transition rate constants in the absence (k_i) and in the presence of modulators (k_{iJ}) were determined by non-linear regression of experimental data to Eq. (1):

$$\frac{e}{e_0} = \left[1 + A\frac{k_{1(J)}}{k_{2(J)} - k_{1(J)}}\right]\exp\left(-k_{1(J)}t\right) - \left[A\frac{k_{1(J)}}{k_{2(J)} - k_{1(J)}}\right]\exp\left(-k_{2(J)}t\right) \quad (1)$$

where A is the molar activity ratio between the intermediate and initial stages, e_0 is the initial activity and e is the residual activity at any time t.

Modulation factors for lactose and galactose were determined from the transition rate constants as [12]:

$$n_{iJ} = 1 - \frac{k_{iJ}}{k_i} \quad (2)$$

where the subscript i denotes the stage and J denotes the modulator.

Temperature-explicit functions for all kinetic and inactivation parameters were derived from the experimental data obtained.

Kinetic rate constants were described by the Arrhenius-type functions:

$$V_{max} = V_{max,0}\exp\left[\frac{-E_a}{RT}\right] \qquad k_{cat} = k_{cat,0}\exp\left[\frac{-E_a}{RT}\right]$$

$$k_{iJ} = k_{iJ,0}\exp\left[\frac{-E_{DiJ}}{RT}\right]$$

$$n_{iJ} = 1 - \frac{k_{iJ,0}}{k_{i,0}}\exp\left[\frac{-(E_{DiJ} - E_{Di})}{RT}\right]$$

Affinity parameters were described by proper equilibrium thermodynamic correlations:

$$K_m = K_{m,0}\exp\left[\frac{\Delta H^0}{RT}\right] \qquad K_P = K_{P,0}\exp\left[\frac{\Delta H_P^0}{RT}\right]$$

All experiments and sample analysis were done in triplicate. Error was less than 5% at 95% level of significance.

3. Reactor modeling for temperature optimization

A scheme for reactor temperature optimization was derived considering the validated temperature-explicit functions for all kinetic and inactivation parameters of CIL. The system under consideration refers to the production of hydrolyzed whey with multiple staggered packed-bed reactors with CIL. Staggering was designed to compensate for enzyme inactivation, obtaining a constant throughput of product to downstream operations at a pre-established small fluctuation in conversion. Optimization was done considering an annual cost objective function, in which the costs of enzyme, reactor (in terms of annual equivalent cost) and utilities (fuel and electricity for pumping, agitation and temperature maintenance) were calculated at different temperatures.

Equations that describe the reactor operation are presented in Appendix A. A computer program for temperature optimization was developed in Visual Basic 5.0, which allows the introduction of variations in all parameters and operational criteria to perform sensitivity analysis. In this case, enzyme replacement criterion, certainly very crucial for reactor optimization, was analyzed.

4. Results and discussion

4.1. Determination of temperature dependency of kinetic and inactivation parameters

Galactose was a competitive inhibitor, but neither glucose nor lactose at high concentration inhibited CIL. Kinetic parameters were obtained from the initial rate data and their values are presented in Table 1. Their logarithms are plotted as a function of inverse temperature in Fig. 1. As seen, Arrhenius functions were validated for k_{cat} ($r = 0.992$) and equilibrium thermodynamic correlations for K_m ($r = 0.995$) and K_P ($r = 0.997$), and the following temperature explicit functions determined:

$$k_{cat} = 1.91 \times 10^7 \exp\left(\frac{-5246.6}{T}\right)$$

$$E_a = 10.43\,[\text{kcal mol}^{-1}]$$

$$K_m = 5.23 \times 10^5 \exp\left(\frac{-2956}{T}\right)$$

$$\Delta H^0 = -5.87\,[\text{kcal mol}^{-1}]$$

$$K_P = 2.66 \times 10^{11} \exp\left(\frac{-6535.7}{T}\right)$$

$$\Delta H_P^0 = -12.99\,[\text{kcal mol}^{-1}]$$

Santos et al. [25] found similar values for E_a and H_P^0 for a yeast lactase but they reported a biphasic mode of dependence for ΔH^0 with a maximum at

Table 1
Kinetic parameters of CIL at pH 6.6

Temperature (°C)	V_{max} (μmol/min g CIL)	k_{cat} (μmol/min IU)	K_m (mM)	K_P (mM)
10	82.9	0.17	15.4	24.7
15	116.1	0.24	18.3	37.0
20	164.6	0.34	21.9	56.1
22.5	186.6	0.39	23.9	69.5
25	211.0	0.44	24.8	80.1
27.5	241.8	0.51	27.5	96.9
30	311.7	0.65	31.7	109.7
32.5	371.0	0.78	33.0	135.1
35	383.0	0.80	37.9	162.0
37.5	415.0	0.88	38.3	200.8
40	474.0	1.00		

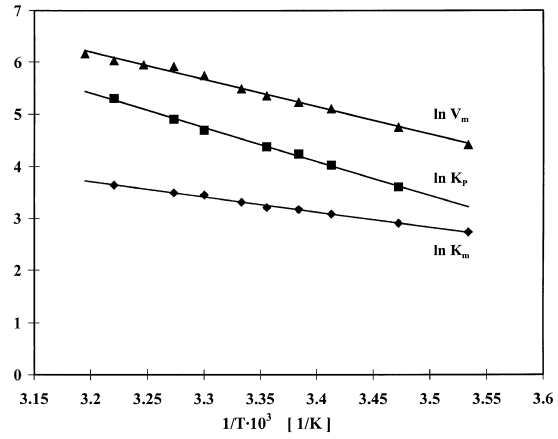

Fig. 1. Temperature dependence of the kinetic parameters of CIL at pH 6.6. ln K_m (mM): ♦; ln K_P (mM): ■; ln V_{max} (μmol/min g): ▲.

25°C. Values reported for fungal lactases indicate milder temperature dependencies than for CIL [26]. The affinity of CIL for lactose and galactose decreased with temperature, as shown by the corresponding correlations for K_m and K_P. The effect is more pronounced for K_P, meaning that from this standpoint, higher temperatures would be beneficial by severely reducing the product inhibition. As shown previously [15], affinity parameters can be considered inherent: chitin is an impervious matrix and external diffusional restrictions are negligible (Damkoehler number below 0.1).

CIL inactivation in the absence of modulators and in the presence of saturating lactose and galactose concentrations was studied in the range of 20–40°C at pH 6.6. This is illustrated in Fig. 2a for CIL in buffer in the whole temperature range and in Fig. 2b for CIL in buffer and in saturating concentrations of lactose and galactose at 27.5°C (similar behavior was obtained at other temperatures). Temperature had a strong effect on CIL inactivation, as illustrated in Fig. 2a, while Fig. 2b illustrates the positive modulation by galactose and negative modulation by lactose, which occurred at all temperatures. Values for the transition rate constants and modulation factors are presented in Table 2. Protection by galactose was stronger and nearly independent of temperature in the first stage of enzyme inactivation, while milder and increasing with temperature on the second stage. Modulation by lactose was negative on both stages, although its effect was much stronger in the second stage, especially at lower temperatures. Correlation coefficients were over 0.95 for the first stage rates but somewhat lower in the second stage, being over 0.9, which is still statistically acceptable.

Logarithms of transition rate constants are plotted as a function of inverse temperature in Fig. 3. As seen, Arrhenius functions were validated in the absence and presence of modulators and the following temperature-explicit functions determined:

$$k_1 = 4.6 \times 10^{17} \exp\left(\frac{-13{,}679}{T}\right)$$

$$E_{D1} = 27.18\,[\text{kcal/mol}]$$

$$k_{1S} = 2.50 \times 10^{17} \exp\left(\frac{-13{,}454}{T}\right)$$

$$E_{D1S} = 26.73\,[\text{kcal/mol}]$$

$$k_{1P} = 3.78 \times 10^{15} \exp\left(\frac{-12{,}750}{T}\right)$$

$$E_{D1P} = 25.33\,[\text{kcal/mol}]$$

$$k_2 = 1.59 \times 10^{21} \exp\left(\frac{-16{,}746}{T}\right)$$

$$E_{D2} = 33.27\,[\text{kcal/mol}]$$

$$k_{2S} = 1.08 \times 10^{4} \exp\left(\frac{-4052}{T}\right)$$

$$E_{D2S} = 8.05\,[\text{kcal/mol}]$$

$$k_{2P} = 1.14 \times 10^{21} \exp\left(\frac{12377}{T}\right)$$

$$E_{D2P} = -24.59\,[\text{kcal/mol}]$$

Temperature correlations were as expected, except for the second stage under galactose modulation where a negative value of E_{D2P} was obtained. Values for E_{DiJ} are in the low range of those reported for enzyme denaturation, but E_{D2P} is outside that range. Parameter A does not depend on modulation [20] and was not a defined function of temperature,

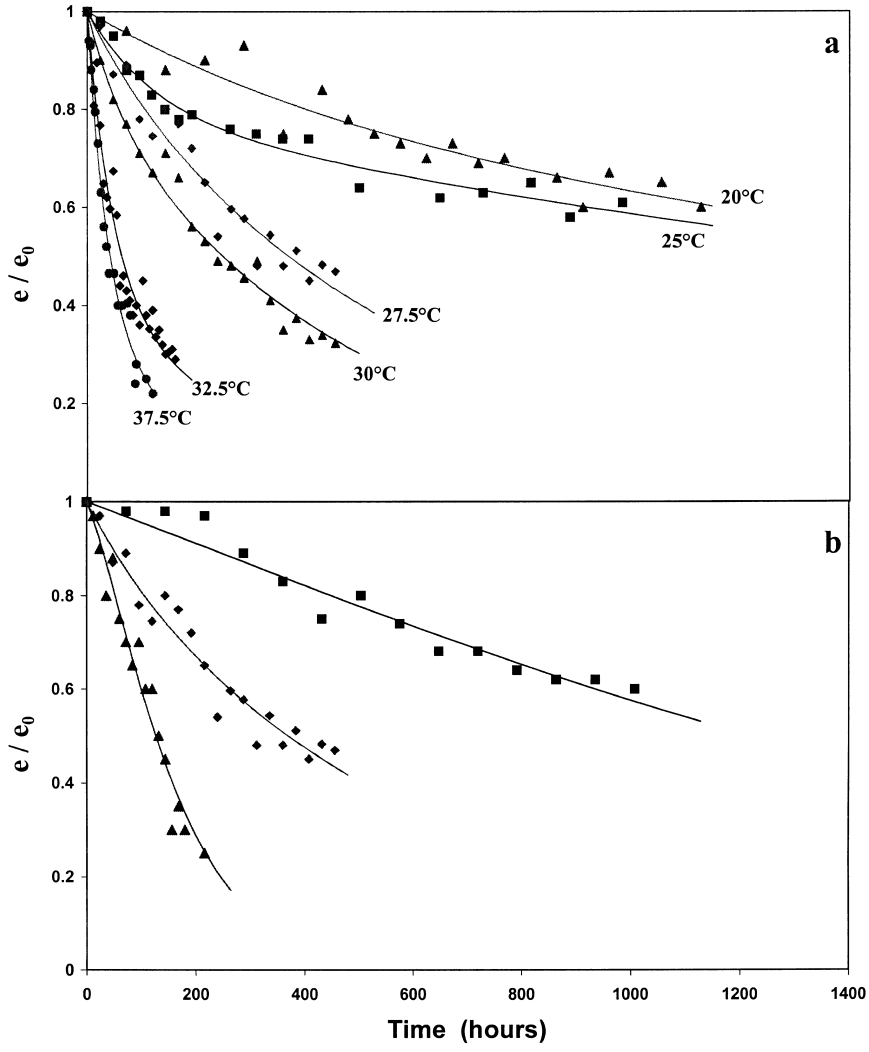

Fig. 2. Time course of CIL inactivation at pH 6.6. (a) In 0.1 M phosphate buffer at different temperatures. (b) At 27.5°C in buffer: ♦; in the presence of lactose concentration ($10 \cdot K_m$): ▲; and in the presence of galactose concentration ($10 \cdot K_P$): ■.

having different values in the 20–30°C and 30–40°C ranges, so that:

$A = 0.70 \qquad T \leq 30°C$

$A = 0.30 \qquad T > 30°C$

From Eq. (2), the temperature functions for modulation factors were:

$$n_{1S} = 1 - 5.43 \times 10^{-1} \exp\left(\frac{225}{T}\right)$$

$$n_{1P} = 1 - 8.22 \times 10^{-3} \exp\left(\frac{929}{T}\right)$$

$$n_{2S} = 1 - 6.79 \times 10^{-18} \exp\left(\frac{12{,}694}{T}\right)$$

$$n_{2P} = 1 - 7.17 \times 10^{-43} \exp\left(\frac{29{,}123}{T}\right)$$

Temperature reduced enzyme affinity for galactose more strongly than for lactose, meaning that the kinetics of CIL is favored as temperature increases. Galactose was a positive modulator, especially in the first stage of CIL inactivation; lactose was a negative modulator, especially in the second stage. Modula-

Table 2
Inactivation parameters of CIL in the absence of modulators (buffer) and in the presence of saturating concentrations of galactose ($10\ K_P$) and lactose ($10\ K_m$)

Parameter	Temperature (°C)					
	20	25	27.5	30	32.5	37.5
$k_1 \cdot 10^3$ (h^{-1})	2.08	5.76	7.57	12.71	19.39	27.06
$k_2 \cdot 10^3$ (h^{-1})	0.30	0.30	1.58	2.14	2.76	4.93
A	0.7	0.7	0.7	0.7	0.3	0.3
r^2	0.92	0.96	0.95	0.98	0.95	0.98
$k_{1P} \cdot 10^3$ (h^{-1})	0.63	0.63	1.44	2.46	2.28	6.59
$k_{2P} \cdot 10^3$ (h^{-1})	1.74	2.37	0.78	0.58	0.50	0.24
r_P^2	0.96	0.86	0.95	0.95	0.98	0.95
$k_{1S} \cdot 10^3$ (h^{-1})	3.57	4.85	9.37	11.27	20.37	42.70
$k_{2S} \cdot 10^3$ (h^{-1})	12.09	11.90	13.73	15.46	21.67	23.59
r_S^2	0.99	0.95	0.96	0.93	0.98	0.96
n_{1P}	0.70	0.89	0.81	0.81	0.88	0.76
n_{2P}	−4.83	−6.9	0.507	0.729	0.819	0.951
n_{1S}	−0.72	0.16	−0.24	0.11	−0.05	−0.58
n_{2S}	−39.30	−38.60	−7.69	−6.22	−6.85	−3.78

r^2: determination coefficient.

tion factors were mild functions of temperature in the first stage, but were strongly temperature-dependent on the second stage of CIL inactivation, where protection by galactose increased with temperature while adverse effect of lactose decreased with temperature. Modulation effect on CIL performance is then more beneficial at higher temperatures, where galactose protection outweighs lactose destabilization. The effect of temperature on modulation factors has not been previously reported, nor temperature functions determined. Inactivation rates have been determined as functions of temperature for fungal lactase but only under non-reactive conditions, so that modulation was not considered [26]. Sugars have been frequently reported as non-specific enzyme stabilizers [27]. This is not the case, however, since glucose and sucrose had no detectable effect on CIL stability, thus, lactose and galactose effects can be properly attributed to catalytic modulation.

4.2. Temperature optimization of continuous packed-bed reactor operation with CIL under modulated inactivation

Continuous packed-bed reactor operation with CIL was simulated to determine optimum temperature by solving the temperature-explicit Eqs. (A2) and (A3). Eq. (A3) takes into consideration the fact that CIL inactivation varies throughout the catalyst bed as a consequence of modulation, being in this case more pronounced near reactor entrance, where lactose negative effect prevails over galactose positive effect, as experimentally proven already [15]. A battery of staggered reactors is required to absorb the fluctuations due to enzyme inactivation. In this case, reactors operate at constant flow-rate during each interval between each reactor start-up (see Appendix A), flow-rate being reduced stepwise after each interval to compensate for enzyme inactivation. Since some enzyme inactivation will occur during that interval,

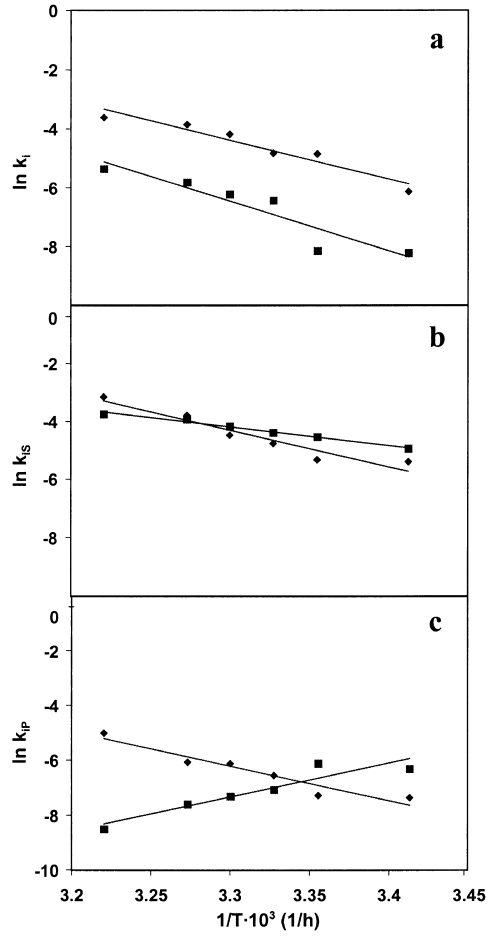

Fig. 3. Temperature dependence of the transition rate constants for CIL inactivation at pH 6.6. (a) In 0.1 M phosphate buffer. (b) In the presence of lactose concentration ($10 \cdot K_m$). (c) In the presence of galactose concentration ($10 \cdot K_P$). k_{1J} (h^{-1}): ◆; k_{2J} (h^{-1}): ■.

certain variation in conversion is expected, which will be smaller with the larger number of reactors. In this case, maximum allowable variation was set as 1% and the number of reactors required to cope with this is 8. One stand-by reactor is required for continuous operation so that the total number of units is 9. Simulation was done under conditions in Table 3; production task equivalent to 3 m^3/h was determined according to a market estimate for the product in Chile [28]. Results are presented in Table 4, where the amount of catalyst (volume of packed-bed), time interval for each reactor start-up and total operating time for each reactor cycle are registered. To determine the annual cost objective function, the cost of CIL was estimated at US$113/kg [28,29]. Costs of fuel and electricity were determined considering the local industrial rates: US$1.51 $\times 10^{-4}$/kcal and US$ 0.125/kW h. Reactors' cost was determined on local quotations from stainless-steel workshops and annual equivalent cost considering a life span of 10 years at 12% annual interest. Considering CIL replacement at 25% residual activity, the results of temperature optimization are summarized in Table 4. Sensitivity analysis with respect to CIL replacement policy is presented as a surface of response in Fig. 4. Optimum temperature was close to 20°C and varied only slightly with catalyst replacement policy, being somewhat higher when the catalyst is replaced at a higher residual activity. Over temperature optimum, cost increases sharply, especially at more frequent catalyst replacement, which reflects the high incidence of the cost of enzyme. Below temperature optimum, cost increase is milder and reflects the incidence of utilities and reactor cost. To evaluate the effect of modulation on reactor behavior, results are presented in Fig. 5 in the presence and absence of modulation, for enzyme replacement at 25% residual activity. As seen, under process conditions, the net effect of modulation is negative, meaning that lactose destabilization prevails over galactose protection. Design under no modulation can then be an unacceptable underestimate. Differences are lower at higher temperatures, where the modulation effect is more beneficial for CIL stability.

Mode of operation implies that variation in product composition can be minimized by increasing the number of reactors. This is certainly a point for further optimization when the quality of product is taken into consideration for process economics. There

Table 3
Conditions of operation for the simulation of continuous packed-bed reactors with CIL

Total flow-rate (m^3/h)	3
Void fraction of CIL bed	0.6
Feed lactose concentration (mM)	146
Specific activity of CIL (IU/g)	350
Final conversion (%)	0.7 ± 0.01
CIL replacement policy (% of initial activity)	5–75
Bed height to diameter ratio	5

Table 4
Temperature optimization for continuous packed-bed reactor operation with CIL considering catalyst replacement at 25% residual activity. CE: cost of enzyme; CU: cost of utilities; CR: equivalent annual cost of reactor; CA: annual cost. All figures are in US$ thousands/year

T (°C)	V_R (m^3)	t_S (h)	t_c (h)	CE	CU	CR	CA
10	0.213	109.4	875	99.2	38.6	10.4	148.2
15	0.149	81.3	650	93.2	19.3	8.6	121.1
20	0.108	53.1	425	103.7	0.4	7.3	111.4
25	0.081	33.8	270	122.4	19.3	6.2	147.9
30	0.063	21.6	173	147.4	38.6	5.4	191.4
35	0.053	10.1	81	265.0	57.9	4.9	327.8
40	0.042	5.7	45.5	373.0	77.2	4.4	454.7

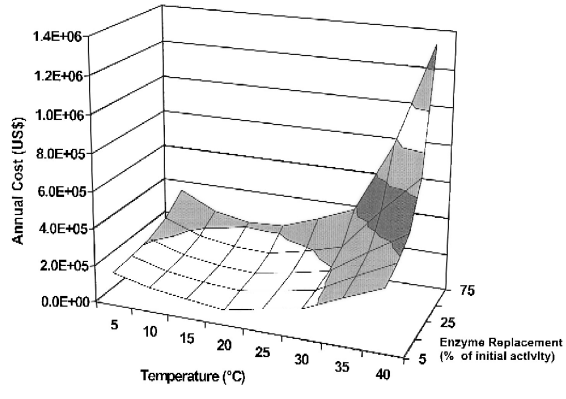

Fig. 4. Surface of response for temperature optimization of continuous staggered packed-bed reactors with CIL. Operational conditions are in Table 3, considering the annual cost of reactor operation as objective function, and enzyme replacement policy as sensitive parameter.

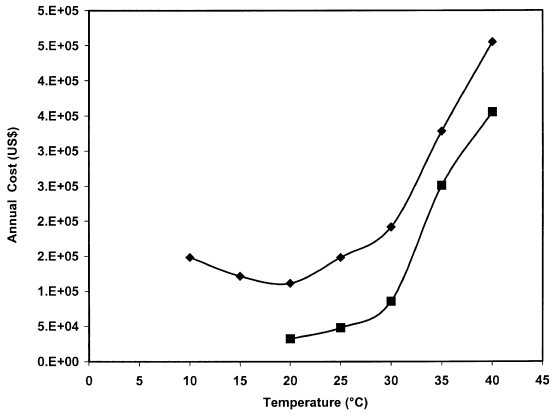

Fig. 5. Temperature optimization of packed-bed reactor with CIL replacement at 25% residual activity considering modulation by lactose and galactose (♦) and not considering modulation (■).

are other strategies for reactor operation to ensure uniform quality at (rather) constant throughput, but they produce unstable operation [19], are hardly applicable for packed-bed reactors [17] or require complex on-line control systems [30] which are not always readily available to the local industry.

5. Conclusions

• Galactose was a competitive inhibitor, while glucose and lactose at high concentration did not inhibit CIL. Galactose was a positive, and lactose, a negative modulator of CIL stability, and CIL inactivation was properly modeled by a two-stage series mechanism.

• Temperature-explicit functions were validated for all kinetic and inactivation parameters of CIL. Values for all kinetic and inactivation parameters increased with temperature. Temperature functions for modulation factors have been reported for the first time.

• Temperature reduced enzyme affinity for galactose more strongly than for lactose, meaning that the kinetics of CIL is favored as temperature increases. Modulation effect on CIL is more beneficial at higher temperatures, where galactose protection outweighs lactose destabilization of CIL. In spite of this, CIL stability decreased dramatically with temperature, which explains the rather low values for temperature optima.

• Optimum temperature for the simulated continuous operation of packed-bed reactors with CIL, considering an annual cost objective function and CIL replacement at 25% residual activity, was 20°C. Optimum temperature varied only slightly with catalyst replacement policy, but the costs were severely affected when the catalyst was recharged at higher than 25% residual activity. Inactivation parameters were not determined below 20°C, so that values used in the simulation are extrapolated. Experiments are underway to determine them at temperature down to 10°C.

• Net effect of modulation was negative, meaning that under the given process conditions, lactose effect was prevalent over galactose protection. This highlights the importance of considering modulation factors for proper reactor design.

• A software for temperature optimization has been developed in Visual Basic 5.0, which is highly flexible, allowing the introduction of variations in rate and inactivation equations, in temperature functions for all parameters, and in operational criteria to perform sensitivity analysis.

Nomenclature

A_S	cross sectional area of reactor
d	total operation time for each reactor cycle
E_a	activation energy of catalyzed reaction
E_{DiJ}	activation energy of inactivation reaction in stage i under modulation by J
e	enzyme activity
F	total flow rate to downstream
F_0	initial feed flow-rate
F_{0i}	initial feed flow-rate to each reactor
ΔH^0	standard enthalpy change of enzyme–lactose complex dissociation reaction
ΔH_P^0	standard enthalpy change of enzyme–galactose complex dissociation reaction
K_m	CIL Michaelis constant for lactose
K_P	CIL competitive inhibition constant for galactose
k_{cat}	catalytic rate constant for lactose hydrolysis
k_{iJ}	transition rate constant of CIL inactivation in stage i under modulation by J
N_R	number of reactors

r	correlation coefficient
R	universal gas constant
s_0	feed lactose concentration
T	temperature
t	time of operation
t_c	time for each cycle
t_s	time interval between each reactor start-up
υ	initial rate of reaction
V_R	volume of each reactor
X	substrate conversion
z	variable height of catalyst bed
ε	void fraction of catalyst bed

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Appendix A. Equations for packed-bed reactor operation with CIL

CIL was inhibited by galactose competitively, so rate equation for lactose hydrolysis with CIL can be represented as:

$$\sigma(X,T) = \frac{\upsilon(X,T)}{k_{cat}(T)\,e} = \frac{s_0(1-X)}{K_m(T)\left[1 + \dfrac{s_0 X}{K_P(T)} + s_0(1-X)\right]} \tag{A1}$$

From a material balance of all enzyme species, according to a modulated two-stage series mechanism and Eqs. (1), (2) and (A1), the enzyme inactivation in the reactor can be described by:

$$-\frac{de}{dt} = e k_1(T)\left[\frac{(1-A(T))\exp(-k_1(T)t)\left[1-\sigma(X,T)N_1(X,T)\right]}{\exp(-k_1(T)t) + \left(k_1(T)\dfrac{A(T)}{(k_2(T)-k_1(T))}\right)\left[\exp(-k_1(T)t) - \exp(-k_2(T)t)\right]}\right.$$
$$\left. + \frac{A(T)k_2(T)\left[\exp(-k_1(T)t) - \exp(-k_2(T)t)\right]\left[1-\sigma(X,T)N_2(X,T)\right]}{(k_2(T)-k_1(T))\exp(-k_1(T)t) + k_1(T)A(T)\left[\exp(-k_1(T)t) - \exp(-k_2(T)t)\right]}\right] \tag{A2}$$

where the generalized modulation factors N_1 and N_2 are defined as:

$$N_1(X,T) = n_{1S}(T) + n_{1P}(T)\frac{K_m(T)X}{K_P(T)(1-X)}$$

$$N_2(X,T) = n_{2S}(T) + n_{2P}(T)\frac{K_m(T)X}{K_P(T)(1-X)}$$

subscipt S stands for lactose and P for galactose.

Material balance for packed-bed reactor with CIL, under pseudo-steady state and plug flow regime yields [12]:

$$\frac{dX}{dz} = \frac{e k_{cat}(T)\sigma(X,T)}{s_0}\frac{\varepsilon A_s}{F_0} \tag{A3}$$

Reactor performance as a function of temperature is described by the numerical solution of differential Eqs. (A2) and (A3). The number of staggered reactors can be determined by considering a maximum allowable variation in product quality (substrate conversion). Time interval between each reactor start-up is:

$$t_s = \frac{d}{N_R}$$

From the enzyme decay curve obtained by solving Eqs. (A2) and (A3), residual enzyme activity after each time interval can be determined, and the flow-rate to each reactor during each interval was calculated as:

$$F_{0i} = \frac{F}{\sum_{i=1}^{N_R} \frac{E_{t=t_s i}}{E_0}}$$

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