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Design of immobilized enzyme reactors for the continuous production of fructose syrup from whey permeate

A. Illanes, L. Wilson, L. Raiman

Abstract Biocatalyst inactivation is inherent to continuous operation of immobilized enzyme reactors, meaning that a strategy must exist to ensure a production of uniform quality and constant throughput. Flow rate can be profiled to compensate for enzyme inactivation maintaining substrate conversion constant. Throughput can be maintained within specified margins of variation by using several reactors operating in parallel but displaced in time. Enzyme inactivation has been usually modeled under non-reactive conditions, leaving aside the effect of substrate and products on enzyme stability. Results are presented for the design of enzyme reactors under the above operational strategy, considering first-order biocatalyst inactivation kinetics modulated by substrate and products. The continuous production of hydrolyzed-isomerized whey permeate with immobilized lactase and glucose isomerase in sequential packed-bed reactors is used as a case study. Kinetic and inactivation parameters for immobilized lactase have been determined by the authors; those for glucose isomerase were taken from the literature. Except for lactose, all other substrates and products were positive modulators of enzyme stability. Reactor design was done by iteration since it depends on enzyme inactivation kinetics. Reactor performance was determined based on a preliminary design considering non-modulated first-order inactivation kinetics and confronted to such pattern. The new pattern of inactivation was then used to redesign the reactor and the process repeated until reactor performance (considering modulation) matched the assumed pattern of inactivation. Convergence was very fast and only two iterations were needed.

List of symbols

A	m^2	cross sectional area of reactor
a	kat/kg	specific activity of catalyst
D	m	reactor diameter
d	s	total operation time for each reactor cycle
E	kat	enzyme activity
e	kat/m^3	volumetric enzyme activity
F	m^3/s	total process flow rate
F_{0i}	m^3/s	initial feed flow-rate to each reactor
H		number of enzyme half-lives used in the reactor
H_b	m	catalyst bed height
H_R	m	reactor height
K_{eq}	kg-mole/m^3	equilibrium constant of glucose isomerization to fructose ($= V_G K_F / V_F K_G$)
K_F	kg-mole/m^3	IGI Michaelis constant for fructose
K_G	kg-mole/m^3	IGI Michaelis constant for glucose
K_L	kg-mole/m^3	CIL Michaelis constant for lactose
K_P	kg-mole/m^3	CIL competitive inhibition constant for galactose
k_{cat}		catalytic rate constant for lactose hydrolysis
k'_{cat}		catalytic rate constant for glucose isomerization
k_D	s^{-1}	first-order inactivation rate constant under no modulation
k_{DJ}	s^{-1}	first-order inactivation rate constant under modulation by J
M	kg	catalyst mass
N_1	–	CIL global modulation factor
N_2	–	IGI global modulation factor
N_R	–	number of reactors
n_L	–	CIL modulation factor by lactose
n_P	–	CIL modulation factor by galactose
n_G	–	IGI modulation factor by glucose
n_F	–	IGI modulation factor by fructose
R_F	–	ratio of minimum to maximum flow rate
s_0	kg-mole/m^3	feed lactose concentration
t	s	time of operation
t_s	s	time interval between each reactor start-up
$t_{1/2}$	s	half life of each catalyst
V_F	mole/m^3·s	maximum reaction rate for fructose isomerization ($= k'_{cat2} \cdot e$)
V_G	mole/m^3·s	maximum reaction rate for glucose isomerization ($= k_{cat2} \cdot e$)
V_b	m^3	volume of catalyst bed
V_R	m^3	volume of reactor
v	mole/m^3·s	initial rate of reaction
X	–	substrate conversion
z	m	variable height of catalyst bed

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ε	–	void fraction of catalyst bed
η'	–	global effectiveness factor of IGI particles
ρ	kg/m^3	apparent density of biocatalyst

Subscripts

1	refers to CIL reactor
2	refers to IGI reactor

1 Introduction

Strategies for continuous operation with immobilized enzyme reactors have been thoroughly reviewed in the literature, biocatalyst inactivation during reactor operation being the key problem to be tackled. Flow rate profiling [1, 2], biocatalyst replacement [3] and temperature profiling [4] have been proposed to compensate for enzyme decay. Biocatalyst replacement is rather straightforward but it has practical limitations, especially when packed-bed reactors are used. Temperature profiling is a rather unstable mode of operation and it is of limited use when enzyme stability is more sensitive to temperature than enzyme activity [5]. In practice, very narrow ranges of temperature can be used [4], which makes operation cumbersome; besides, proper reactor design requires of temperature explicit functions for kinetic [6] and inactivation [7] parameters. The first strategy is based on compensating enzyme decay by decreasing flow rate, since to keep substrate conversion (product quality) constant, the ratio of enzyme activity to flow rate must remain constant. Variable throughput is undesirable for downstream operations, but total flow rate can be held within a limited margin of variation by using a staggered multiple reactor system, each reactor working at a varying flow rate [8]. This strategy of operation is increasingly interesting as on-line measurement and computer control are now becoming common to industrial operation, and will be used in this work.

Enzyme inactivation is a very complex process and several mechanisms have been proposed to describe it [7, 9, 10]. Models based on complex mechanisms contain a number of parameters that exceed the possibility of their experimental determination; therefore, models based on simple first-order kinetics or two-stage series mechanisms have been frequently used, despite its obvious simplification [11, 12, 13]. Reactor design considering enzyme inactivation frequently disregard the effect of substrates and products on inactivation rate constants [5, 14, 15, 16] or is based on global inactivation rate constants determined under particular operating conditions [3, 6, 17], not allowing to discriminate the individual modulation effects. Only in a few cases, protection by substrate has been evaluated and considered for reactor design [2, 18, 19, 20] and, even in fewer, product modulation has been included [21, 22]. In the case of the enzymes considered in this work, the modulation effects of substrates and products have been clearly established [22, 23], so that reactor design will consider such effects. This is the first case in which packed-bed reactors are designed considering both substrate and product modulation on enzyme inactivation.

This work presents a design scheme for a system of staggered packed-bed enzyme reactors with immobilized lactase and glucose isomerase, within an industrial plant designed for the production of hydrolyzed-isomerized whey permeate [24]. Data for immobilized lactase from *Aspergillus oryzae* has been obtained by the authors [25], while data for immobilized glucose isomerase have been taken from the literature [18, 23].

2 System description

The process under consideration refers to the enzymatic production of 61% solids hydrolyzed-isomerized whey permeate syrup (HIPS), by the sequential use of packed-bed reactors with immobilized lactase and glucose isomerase. The production of hydrolyzed whey permeate has been evaluated as commercially feasible [26], despite its sweetness is substantially lower than sucrose. This problem can be overcame by coupling the process to an enzymatic isomerization step; however, hydrolyzed-isomerized permeate has seldom been considered despite its interesting properties [27]. Its sweetness in dairy products has been determined as 90% with respect to sucrose syrup of equal solids concentration [28], therefore having several potential applications in the dairy industry. Besides, the process for HIPS production uses surplus whey as raw material, which is relevant from an environmental perspective. Whey permeate, obtained by ultrafiltration is pre-concentrated to 10% solids and lactose hydrolyzed at pH 4.0 and 40 °C with immobilized lactase to a 90% conversion. This stream is then concentrated to 40% solids and subjected to isomerization at pH 7.2 and 60 °C with immobilized glucose isomerase to a 45% conversion. This stream is then concentrated and refined to produce HIPS [24].

Both reactions are conducted in staggered multiple packed-bed reactors, considering 10 and 13% as maximum variations in total flow-rate and catalyst replacement at 20% residual activity. Operation is designed to produce 8170 tons/yr of HIPS by processing 15.9 tons/h of whey, which represents 90% of whey surplus of two main cheese producers in Chile [24].

3 Mathematical model for reactor design

3.1 Enzyme kinetics

Chitin immobilized lactase (CIL) is competitively inhibited by galactose, while glucose has no effect on enzyme activity. Values for kinetic parameters at operating pH and temperature are in Table 1 [29]. Chitin is an impervious material [30] and external diffusional restrictions are negligible [25], so that kinetic parameters can be considered inherent. Rate expression for lactose hydrolysis with CIL can be represented in terms of conversion as:

$$\nu(X_1) = \frac{k_{\mathrm{cat1}}\ e_1\ s_0(1 - X_1)}{K_{\mathrm{L}}\left(1 + \frac{s_0\ X_1}{K_{\mathrm{p}}}\right) + s_0(1 - X_1)} \quad .$$

Isomerization of glucose into fructose with immobilized glucose isomerase (IGI) can be described by reversible

Table 1. Kinetic and inactivation parameters for CIL, at 40°C and pH 4.0, and IGI, at 60°C an pH 7.5

CIL		Ref.	IGI		Ref.
a_1 [kat/kg]	$4.56 \cdot 10^{-3}$	29	a_2 [kat/kg]	$4.8 \cdot 10^{-3}$	2
			a_2' [kat/kg]	$3.12 \cdot 10^{-3}$	2
K_L [mol/m^3]	91	29	K_G [mol/m^3]	$7 \cdot 10^2$	2
K_P [mol/m^3]	14.7	29	K_F [mol/m^3]	$4.5 \cdot 10^2$	2
			K_{eq} [mol/m^3]	1	2
k_{D1} [s^{-1}]	$6.67 \cdot 10^{-7}$	31	k_{D2} [s^{-1}]	$2.78 \cdot 10^{-7}$	2
n_L	−0.35	11	n_G	0.5	18
n_P	0.48	11	n_F	0.5	18
			η'	0.8	2

Michaelis-Menten kinetics [16]. Values for kinetic parameters at operating pH and temperature are in Table 1 [2]. Effectiveness factor for IGI particle was only slightly dependent on bulk glucose concentration and a constant value of 0.8 has been assumed [2]. Rate expression for glucose isomerization with IGI can be represented in terms of conversion as:

$$v(X_2) = \eta' \frac{V_{map} \cdot g_0 \ (X_{eq} - X_2)}{K_{map} + g_0(X_{eq} - X_2)} \ ,$$

where:

$$V_{map} = \frac{V_G \cdot K_F}{K_F - K_G} \cdot \left(1 + \frac{1}{K_{eq}}\right) \ ,$$

$$K_{map} = \frac{K_G \cdot K_F}{K_F - K_G} \left[1 + \left(\frac{1}{K_G} + \frac{1}{K_F}\right) \cdot \frac{g_0}{1 + K_{eq}}\right] \ .$$

3.2 Enzyme inactivation

Lactose and galactose affect lactase stability being negative and positive modulators respectively. Glucose and fructose have proven to be positive modulators of IGI stability. Therefore enzyme inactivation kinetics expressions were derived considering such modulation effects.

Modulation factors have been defined as [21]:

$$n_J = 1 - \frac{k_{DJ}}{k_D} \ .$$

Lactase inactivation considering modulation by lactose and galactose was modeled as simple first-order kinetics, as described by Eq. (1) [11]:

$$-\frac{dE_1}{dt} = E_1 \cdot k_{D1} \cdot [1 - \sigma(X_1) \cdot N_1(X_1)] \ , \qquad (1)$$

where:

$$\sigma(X_1) = \frac{v(X_1)}{k_{cat1} e_1} = \frac{s_0(1 - X_1)}{K_L\left(1 + \frac{S_0 X_1}{K_P}\right) + s_0(1 - X_1)} \ ,$$

$$N_1(X) = n_L + n_P \frac{K_L \cdot X_1}{K_P(1 - X_1)} \ .$$

Glucose isomerase inactivation considering modulation by glucose and fructose was modeled as simple first-order kinetics, as described by Eq. (2) [2]:

$$-\frac{dE_2}{dt} = E_2 \cdot k_{D2} \cdot [1 - N_2(X_2)] \ , \qquad (2)$$

where:

$$N_2(X_2) = 1 - \frac{K_G + n_G \cdot g_0(1 - X_2) + n_F \cdot g_0 X_2 \cdot \frac{K_G}{K_F}}{K_G + g_0(1 - X_2) + g_0 X_2 \cdot \frac{K_G}{K_F}} .$$

3.3 Reactor design

3.3.1 Determination of the number of reactors

The number of reactors required in each reaction step can be determined according to [1]:

$$N_R = -H \frac{\ln 2}{\ln R_F} \ . \qquad (3)$$

Values of H and R_F for the hydrolysis and isomerization steps are in Table2.

3.3.2 Determination of initial flow rate to each reactor

Operation time for each reactor will be:

$$d = H \cdot t_{1/2} \ . \qquad (4)$$

Initially, non-modulated first order kinetics of enzyme inactivation will be assumed, so that:

$$t_{1/2} = \frac{\ln 2}{k_D} . \qquad (5)$$

Values of k_D for CIL and IGI are in Table 2.

Time interval between each reactor start-up, according to the staggered mode of operation, will be:

Table 2. Operation parameters for CIL and IGI packed-bed reactors

CIL		Ref.	IGI		Ref.
H_1	2.32	24	H_2	2.32	24
R_F	0.818	24	R_F	0.77	24
F [m^3/s]	$2.19 \cdot 10^{-3}$	24	F [m^3/s]	$5.09 \cdot 10^{-4}$	24
X_1	0.9	24	X_2	0.45	24
s_0 [mol/m^3]	$2.86 \cdot 10^2$	24	g_0 [mol/m^3]	$1.11 \cdot 10^3$	24
ρ_1 [kg/m^3]	0.18	25	ρ_2 [kg/m^3]	0.335	2

$$t_s = \frac{d}{N_R} \quad . \tag{6}$$

Flow rate to each individual reactor will be profiled to follow enzyme inactivation (initially assumed as exponential), so that total process flow rate in any given time will be:

$$F = \sum_{i=1}^{N_R} F_i = F_{0i} \cdot \sum_{i=1}^{N_R} \exp[-k_D \cdot t_s \cdot i] \quad . \tag{7}$$

Values of F, as determined from the material balance of the plant, are in Table 2. Then F_{0i} can be calculated from Eq. (7).

3.3.3 Preliminary reactor design

The amount of catalyst to be charged in each reactor can be determined from the corresponding equations for steady state operation of CIL and IGI reactors:

$$\text{CIL} \quad \frac{k_{cat1} \cdot E_1}{F_{0i} \cdot K_L} = \frac{M_1 \cdot a_1}{F_{0i} \cdot K_L} = s_0 \cdot X_1 \left(\frac{1}{K_L} - \frac{1}{K_P}\right) - \left(1 + \frac{s_0}{K_P}\right) \times \ln(1 - X_1) \quad , \tag{8}$$

$$\text{IGI} \quad \eta' \frac{k_{cat2} \cdot E_2}{F_{0i} \cdot K_{map}} = \eta' \frac{M_2 \cdot a_2}{F_{0i} \cdot K_{map}} = \left[\frac{g_0 \cdot X_2}{K_{map}} - \ln\left(\frac{X_{eq} - X_2}{X_{eq}}\right)\right] \cdot \frac{V_G}{V_{map}} \tag{9}$$

Volume of catalyst bed in each reactor can then be determined as:

$$V_b = \frac{M}{\rho} \quad , \tag{10}$$

and reactor dimensions determined from V_b using geometric and hydrodynamic criteria, as presented elsewhere [2].

3.3.4 Reactor performance under modulated thermal inactivation

Material balance for CIL and IGI packed-bed reactors under plug-flow regime and pseudo-steady state operation will yield:

$$\text{CIL} \quad \frac{dX_1}{dz} = \frac{k_{cat1} \cdot E_1(t) \cdot \sigma(X) \cdot A_1}{V_b \cdot F_{0i} \cdot s_0} \quad , \tag{11}$$

$$\text{IGI} \quad \frac{dX_2}{dz} = \frac{\frac{k_{cat2} \cdot E_2(t) \cdot K_G (1 + \frac{1}{K_{eq}})(X_{eq} - X_2)}{V_b (K_F - K_G)}}{K_{map} + g_0 (X_{eq} - X_2)} \cdot \frac{\eta' \cdot A_2}{F_{0i}} \tag{12}$$

Reactor performance under modulation will be described by the resolution of Eqs. (1) and (11) for CIL reactor and (2) and (12) for IGI reactor, using the preliminary design data. The system of differential equations was solved numerically using the modified Euler method and computer resolution was done in Visual Basic 3.0 for Windows. Modulation of enzyme inactivation will produce a differential decay through the catalyst bed according to the profiles of modulators through it, which has been experimentally confirmed [22]. The resolution will yield enzyme activity profiles with time at constant pseudo-steady state conversion. From the enzyme inactivation profile, a new operational half-life will be determined, and new values for d and t_s will be obtained from Eqs. (4) and (6). Kinetics of enzyme inactivation (and as a consequence, flow rate profile) will not be necessarily exponential, as originally assumed in Eq. (7). However, from the enzyme decay curve considering modulation, flow rate can be profiled as:

$$F = \sum_{i=1}^{N_R} F_i = F_{0i} \cdot \sum_{i=1}^{N_R} r_{Fi} \quad , \tag{13}$$

where

$$r_{Fi} = \frac{F_{t=t_s \cdot i}}{F_{0i}} \quad . \tag{14}$$

The values of F_{0i} for CIL and IGI reactors obtained from Eq. (13), are now used in Eqs. (8) and (9) respectively, to determine the new catalyst charge, catalyst bed volume and reactor dimensions, as in section 3.3.3. Reactor performance is determined from the new design data and the procedure is repeated until the system converges, i.e. the calculated pattern of enzyme inactivation matches the one previously assumed.

4 Results

Reactor design is illustrated for the case of an industrial plant for the production of HIPS in Chile, using 90% of whey surplus of two main cheese producers as raw material. Market for HIPS and the protein recovered by ultrafiltration exceed demand [24]. Plant throughput is equivalent to 15.9 tons/h of whey.

Kinetic and inactivation parameters for CIL and IGI are in Table 1. Operation parameters for CIL and IGI reactor design are in Table 2. The number of reactors is calculated from Eq. (2) and is presented in Table 3.

Table 3. Results for preliminary reactor design, not considering modulation effects by substrate or product

CIL		Eq.	IGI	Eq.
N_R	8	3	6	3
d [s]	$2.42 \cdot 10^6$	4	$5.79 \cdot 10^6$	4
t_s [s]	$3.02 \cdot 10^5$	6	$9.63 \cdot 10^5$	6
F_{0i} [m^3/s]	$6.09 \cdot 10^{-4}$	7	$1.95 \cdot 10^{-4}$	7
M [kg]	393.56	8	210.31	9
V_b [m^3]	2.19	10	0.63	10
V_R [m^3]	2.74		0.78	
D [m]	0.82		0.54	
H_b [m]	4.14		2.72	
H_R [m]	5.18		3.40	
A [m^2]	0.53		0.23	

4.1
Preliminary reactor design

Results for preliminary reactor design, considering enzyme inactivation under no modulation, are presented in Table 3. Values for operation time and time interval for reactor start-up were calculated from Eqs. (4) and (6). Initial flow rates to each reactor were calculated from Eq. (7) and the amounts of CIL and IGI required were calculated from Eqs. (8) and (9) respectively. Volumes of catalyst beds for each reactor were calculated from Eq. (10); reactor dimensions were determined as indicated in section 3.3.3.

4.2
Reactor performance and design under modulated thermal inactivation

Reactor performance under modulation is determined by solving differential equations, Eqs. (1) and (11) for CIL reactor and (2) and (12) for IGI reactor, using the preliminary design data in Table 3. Results for reactor performance are presented in terms of enzyme inactivation pattern at constant pseudo steady-state substrate conversion at reactor outlet in Figs. 1 and 2 for CIL and IGI respectively. Operation of a single reactor is presented in each case. Data are taken from Tables 1 and 2.

Enzyme inactivation profiles differ from the assumed non-modulated first-order kinetics (thick and thin lines respectively in Figs. 1 and 2), so that new values of enzyme half-lives can be obtained from them, as presented in Table 4. New values for operation time and time interval for reactor start-up were calculated from Eqs. (4) and (6). Initial flow rates to each reactor were now calculated from Eq. (13), which represents the flow rate profiles corresponding to enzyme inactivation profiles considering modulation. The corrected amounts of CIL and IGI required were calculated from Eqs. (8) and (9) respectively. Corrected volume of catalyst beds for each reactor was calculated from Eq. (10) and new reactor dimensions were determined as indicated in section 3.3.3. Summary of results for reactor design is presented in Table 4. From this data, reactor performance was again determined, now matching the assumed pattern of enzyme inactivation represented by thick lines in Figs. 1 and 2, which validates reactor design. Operation of staggered multiple packed-bed CIL and IGI reactors is presented in Fig. 3.

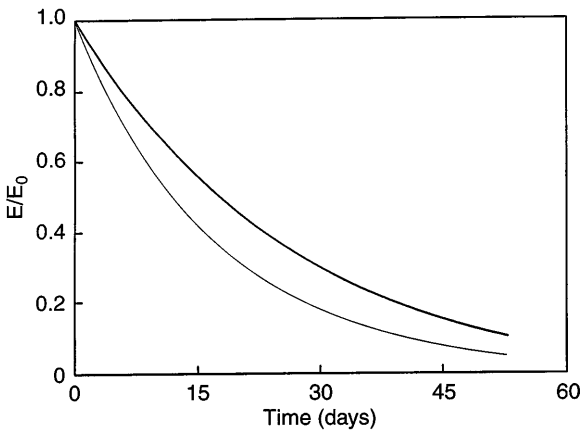

Fig. 1. Enzyme inactivation pattern for continuous packed-bed reactor with CIL at 40°C and pH 4.0 at 0.9 constant conversion. (——): considering modulation by lactose and galactose; (_____): not considering modulation

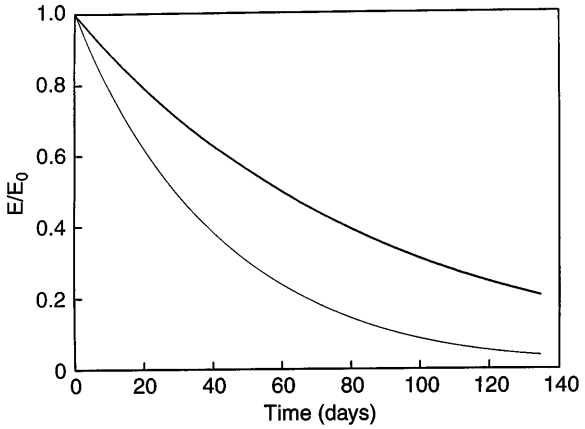

Fig. 2. Enzyme inactivation pattern for continuous packed-bed reactor with IGI at 60 °C and pH 7.2 at 0.45 constant conversion. (——): considering modulation by glucose and fructose; (_____): not considering modulation

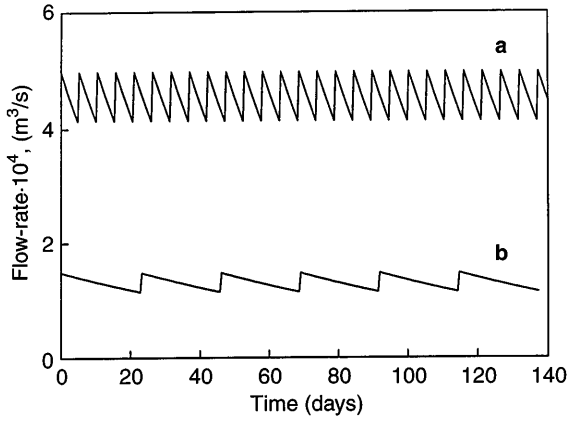

Fig. 3. Performance of staggered multiple packed-bed reactors with CIL (a) and IGI (b) for constant conversion of 0.9 and 0.45 respectively

Table 4. Results for reactor design, considering modulation effects by substrate and product

CIL		Eq.	IGI	Eq.
N_R	8	3	6	3
d [s]	$3.50 \cdot 10^6$	4	$1.19 \cdot 10^7$	4
t_s [s]	$4.38 \cdot 10^5$	6	$1.98 \cdot 10^6$	6
F_{0i} [m^3/s]	$4.97 \cdot 10^{-4}$	13	$1.48 \cdot 10^{-4}$	13
M [kg]	321.52	8	159.21	9
V_b [m^3]	1.79	10	0.47	10
V_R [m^3]	2.23		0.59	
D [m]	0.77		0.49	
H_b [m]	3.82		2.48	
H_R [m]	4.78		3.10	
A [m^2]	0.47		0.19	

5
Discussion and conclusions

Flow rate profiling to compensate for enzyme inactivation is a useful alternative for continuous enzyme reactor operation at constant substrate conversion. Its main drawback is variable throughput, that can be overcome by using staggered multiple reactors. A thorough model for enzyme inactivation is essential to proper design in this mode of operation. Usually, first-order kinetics of inactivation under non-reactive conditions has been considered, neglecting the modulation effect of substrates and products on enzyme stability. Results clearly demonstrate the effect of substrate and product modulation on enzyme reactor design. Such modulation effects, as can be seen from results in Figs. 1 and 2, significantly affect reactor operation. Results in Tables 3 and 4 show that the amount of catalyst and reactor volume required considering modulation, are only 82% , for CIL, and 75% , for IGI, of the corresponding values calculated not considering such effects. The effect of modulation on reactor design is more pronounced for IGI reactor, which is a consequence of the protection effect exerted both by substrate and product. For CIL reactor, the effect on design, although considerable, is lower than in IGI reactor because of the negative effect of lactose on enzyme stability, which partially counteracts the protection exerted by galactose. Inactivation kinetics, at least for CIL, have proven to be better modeled by two-stage series type inactivation [32], so that reactor design can be refined by replacing Eq. (1) by such model. Experiments are underway to determine the increased number of parameters required. The proposed scheme for reactor design can accommodate any model for enzyme kinetics and enzyme inactivation. Reactor design considering the combined modulation effect by substrates and products has not been reported before, being a contribution for more consistent reactor design.

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