OPTIMAL DESIGN OF A SERIES OF CSTR'S FOR BIOCHEMICAL REACTIONS IN THE PRESENCE OF ENZYME DEACTIVATION

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Introduction

Some attention has been given to reactor systems consisting of a series of continuous stirred-tank reactors (CSTR's) for the performance of biochemical reactions of industrial interest. An example of such a process is the production of modified milk for lactose-intolerant people. Food-grade preparations of crude lactase from microbial origin are added to skim milk in a cascade of stirred tanks operated continuously to effect the hydrolysis of lactose into its constituent monosaccharides (glucose and β -galactose) prior to pasteurization. Interest has arisen due to the simplicity of the mathematical simulation of a CSTR (due to the absence of both concentration and temperature gradients within the reacting fluid) and the practical importance of such systems in the food field (provided that large flow rates of fluid raw materials are handled, the products thereby obtained are subject to narrow market specifications, the cost of enzyme makeup is not very high, the level of purity of the enzyme with respect to other inert species is not critical for efficient operation, and the residual enzymatic activity in the effluent stream can be easily destroyed via thermal treatment or otherwise⁷).

Theoretical reasonings underlying the optimal design of a series of CSTR's performing chemical reactions that obey the power law^{1, 5}) and Michaelis-Menten kinetic equation^{2, 4, 6, 9, 10, 11}) are available in the literature. The latter analyses all assume a constant activity of the enzyme along the reactor network and throughout time. Unlike most organic and inorganic reactions effected by homogeneous catalysis, however, enzyme-catalyzed biochemical reactions are subject to thermal deactivation of the biocatalyst due to protein irreversible denaturation or detrimental chemical modification of amino acid residues³).

The purposes of this Short Communication are to (i) derive a general relation that must be obeyed by the intermediate substrate concentrations in order to the overall volume of the reactor network be a minimum, (ii) develop a corresponding approximate, yet much simpler relation,

and (iii) discuss the goodness of such an approximation for cases with physical interest.

Mathematical Analysis

Malcata¹²) has shown elsewhere that the steady-state mass balance to substrate undergoing a biochemical reaction following Michaelis-Menten kinetics and performed in a cascade of isothermal CSTR's in the presence of first-order enzyme deactivation may be written as

$$C_{s,i-1}^* - C_{s,i}^* = \frac{Da_i C_{s,i}^*}{(K^* + C_{s,i}^*) \prod_{j=1}^i (1 + \eta Da_j)} \quad (1)$$

$$i = 1, 2, \dots, N-1$$

where the normalized concentration of substrate, C_s^* , and the dimensionless parameters Da , K^* , and η are defined in the Nomenclature.

Solving Eq. (1) for Da_i for a generic i , and applying the obtained recursive relation for Da_j with $1 \leq j < i$, one obtains after some algebraic manipulation

$$Da_i = \frac{(C_{s,i-1}^* - C_{s,i}^*)(K^* + C_{s,i}^*)}{C_{s,i}^*} \cdot \frac{1}{1 - \eta \sum_{j=1}^i \frac{(C_{s,j-1}^* - C_{s,j}^*)(K^* + C_{s,j}^*)}{C_{s,j}^*}} \quad (2)$$

$$i = 1, 2, \dots, N-1$$

where $C_{s,0}^* = 1$ by definition.

Previously it was proved that there is a minimum concentration of substrate at the outlet stream from the N -th reactor¹²). This situation of maximum conversion of substrate (which corresponds to an overall infinite reactor volume) is obtained when the concentration of substrate at the outlet of every reactor (except the last in the series) is the geometric mean of the concentrations of substrate at the outlets of the previous and next reactors in the cascade. The problem now arises as to calculation of the intermediate concentrations of substrate which lead to the minimum overall volume of the cascade of CSTR's for a known overall conversion. Mathematically

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stated, this corresponds to

$$\left\{ \frac{\partial \sum_{i=1}^N Da_i}{\partial C_{S,j}^*} \right\}_{\eta, K^*, C_{S,i}^*, C_{S,i+1}^*} = 0, j = 1, 2, \dots, N-1 \quad (3)$$

From careful inspection of Eq. (2), one realizes that $Da_i \equiv Da_i(\eta, K^*; C_{S,1}^*, C_{S,2}^*, \dots, C_{S,i-1}^*, C_{S,i}^*)$ ($1 < j < N$). Hence Eq. (3) becomes

$$\sum_{i=j}^N \left\{ \frac{\partial Da_i}{\partial C_{S,j}^*} \right\}_{\eta, K^*, C_{S,i}^*, C_{S,i+1}^*} = 0, j = 1, 2, \dots, N-1 \quad (4)$$

Employing the rules of differentiation to Eq. (4), one obtains after some algebraic work

$$\begin{aligned} & \frac{1 + K^* \frac{C_{S,i-1}^*}{C_{S,i}^{*2}}}{1 - \eta \sum_{j=1}^i \left(\frac{(C_{S,j-1}^* - C_{S,j}^*)(K^* + C_{S,j}^*)}{C_{S,j}^*} \right)} \dots \\ & \dots \left[1 + \frac{\eta \frac{(C_{S,i-1}^* - C_{S,i}^*)(K^* + C_{S,i}^*)}{C_{S,i}^*}}{1 - \eta \sum_{j=1}^i \left(\frac{(C_{S,j-1}^* - C_{S,j}^*)(K^* + C_{S,j}^*)}{C_{S,j}^*} \right)} \right] \dots \\ & + \left(\left(1 + \frac{K^*}{C_{S,i+1}^*} \right) \dots \right. \\ & \dots \left(1 - \eta \left(\sum_{j=1}^i \left(\frac{(C_{S,j-1}^* - C_{S,j}^*)(K^* + C_{S,j}^*)}{C_{S,j}^*} \right) \right. \right. \\ & \quad \left. \left. + (C_{S,i}^* - C_{S,i+1}^*) \left(1 + K^* \frac{C_{S,i-1}^*}{C_{S,i}^{*2}} \right) \right) \right) \right) \\ & + \frac{\left(1 - \eta \sum_{j=1}^{i+1} \left(\frac{(C_{S,j-1}^* - C_{S,j}^*)(K^* + C_{S,j}^*)}{C_{S,j}^*} \right) \right)^2 \dots}{\left(1 - \eta \left(\left(1 + \frac{K^* C_{S,i-1}^*}{C_{S,i}^{*2}} \right) - \left(1 + \frac{K^*}{C_{S,i+1}^*} \right) \right) \dots \right.} \\ & \left. - \sum_{k=2}^{N-i} \frac{\dots (C_{S,i+k-1}^* - C_{S,i+k}^*)(K^* + C_{S,i+k}^*)}{C_{S,i+k}^* \left(1 - \eta \sum_{j=1}^{i+k} \left(\frac{(C_{S,j-1}^* - C_{S,j}^*)(K^* + C_{S,j}^*)}{C_{S,j}^*} \right) \right)^2} \right) \\ & = 0 \end{aligned} \quad (5)$$

The solution of Eq. (5) (i.e., $C_{S,i}^* = C_{S,i}^*, \text{opt}$, $1 \leq i < N$) is thus a complex function of all intermediate concentration. For the case of $\eta = 0$, however, the final solution takes the simple form⁵⁾

$$C_{S,i}^*, \text{opt} = C_{S,N}^{*i/N} \quad (6)$$

Combining Eq. (6) with Eq. (2), and recalling the definition of Da_{tot} , one obtains

$$Da_{\text{tot, opt}} \sim \sum_{i=1}^N \frac{K^* + C_{S,N}^{*i/N}}{\left(\frac{1}{C_{S,N}^{*-1/N-1}} \right) - \eta \sum_{j=1}^i (K^* + C_{S,N}^{*j/N})} \quad (7)$$

where the above value of $Da_{\text{tot, opt}}$ is an approximation to the minimum overall Damköhler number of the reactor network in the presence of enzyme deactivation if the assumption of negligible deactivation is satisfied (i. e., if the ratio of the time constant associated with deactivation of enzyme, $1/k_d$, to the time constant associated with

flow through the reactor network, $\left(\sum_{i=1}^N V_i \right)/Q$, is very large, or, equivalently, if $1/(\eta \cdot Da_{\text{tot}}) \gg 1$).

Recalling the properties of the sum of terms of a geometric series, Eq. (7) can be transformed into

$$\begin{aligned} Da_{\text{tot, opt}} \sim \sum_{i=1}^N \frac{(1 - C_{S,N}^{*1/N})(K^* + C_{S,N}^{*i/N})}{(1 - \eta) C_{S,N}^{*1/N} - \eta K^* (1 - C_{S,N}^{*1/N}) i + \dots} \\ \dots \eta C_{S,N}^{*1/N} C_{S,N}^{*i/N} \end{aligned} \quad (8)$$

The values of $Da_{\text{tot, opt}}$ calculated from Eq. (8) can be compared with the true values obtained by combination of Eqs. (2) and (5) in order to evaluate the accuracy of the approximation. For a large number of reactors in the series (say, $N > 4$), the true value of $Da_{\text{tot, opt}}$ in the presence of deactivation tends asymptotically toward that obtained via a mass balance to a plug-flow reactor under the same conditions of operation, viz⁸⁾.

$$Da_{\text{tot, } N \rightarrow \infty} = -\frac{1}{\eta} \ln \{ 1 + \eta (K^* \ln \{ C_{S,N}^* \} + C_{S,N}^* - 1) \} \quad (9)$$

The results of an exhaustive search for the optimum intermediate concentrations (using incremental steps of 0.001) leading to the true value of $Da_{\text{tot, opt}}$ followed by calculation of the associated ratio of time scales of deactivation and flow through the reactor cascade, for $0.01 < K^* < 10$ (fractional increments of 10), $0.01 < \eta < 1$ (fractional increments of 10), $2 \leq N \leq 4$ (increments of unity), and three outlet concentrations ($C_{S,N}^* = 0.10$, $C_{S,N}^* = 0.50$, and $C_{S,N}^* = 0.90$) are tabulated in **Tables 1 to 3**. The corresponding absolute fractional deviations of these true values from the approximate values of $Da_{\text{tot, opt}}$ (calculated for negligible deactivation), are listed in **Tables 4 to 6** for the same parameter ranges as above. (Although all such values were employed in computation of the average and maximum percent deviations as reported below, only those values which satisfy the condition $1/(\eta \cdot Da_{\text{tot, opt}}) \gg 1$ and do not violate physical constraints¹²⁾ have been tabulated.)

Discussion

As expected, the overall volume of the cascade of

Table 1. Values of $Da_{\text{tot,opt}}$ (first row) and $1/(\eta \cdot Da_{\text{tot,opt}})$ (second row) for various numbers of reactors and values of parameter K^* , assuming that $\eta = 0.01$, for $C_{S,N}^* = 0.1, 0.5$, and 0.9 (first, second, and third set of rows respectively).

K^*	$N = 2$	$N = 3$	$N = 4$	$N = \infty$
1	5.439	4.495	4.117	3.255
	18.386	22.247	24.290	30.722
0.1	1.346	1.257	1.221	1.137
	74.294	79.554	81.900	87.951
0.01	0.951	0.941	0.937	0.927
	105.152	106.270	106.724	107.875
10	9.409	8.789	8.502	7.722
	10.628	11.378	11.762	12.950
1	1.342	1.291	1.267	1.200
	74.516	77.459	78.927	83.333
0.1	0.585	0.580	0.578	0.571
	170.940	172.414	173.010	175.131
0.01	0.510	0.510	0.509	0.508
	196.078	196.078	196.464	196.850
10	1.192	1.183	1.176	1.160
	83.893	84.531	85.034	86.207
1	0.209	0.208	0.207	0.206
	478.469	480.769	483.092	485.437
0.1	0.111	0.111	0.111	0.111
	900.901	900.901	900.901	900.901
0.01	0.101	0.101	0.101	0.101
	990.099	990.099	990.099	990.099

reactors decreases as the number of reactor units is increased towards the value arising from approximate plug flow behavior (cf. Table 1 to 3). The plug-flow model is a good representation of the behavior of the cascade of CSTR's for N , of, say, above 4, especially for small overall conversions and small values of parameter K^* (i.e., pseudo-zero order differential behavior). For these conditions, the ratio $1/(\eta \cdot Da_{\text{tot,opt}})$, which is a measure of deviation from the assumption of negligible deactivation, is approximately proportional to $1/\eta$.

Inspection of the values of percent deviations (most of which are listed in Tables 4 to 6), one concludes that the average percent deviation between the true value of $Da_{\text{tot,opt}}$ and its approximate counterpart is $\pm 0.17\%$ for a number of reactors between 2 and 4 (in practice, the number of reactors rarely exceeds four). The maximum deviation is ca. 5%. The largest errors tend to occur at substrate conversions in the vicinity of the upper limit

Table 2. Values of $Da_{\text{tot,opt}}$ (first row) and $1/\eta \cdot Da_{\text{tot,opt}}$ (second row) for various numbers of reactors and parameter K^* , assuming that $\eta = 0.1$, for $C_{S,N}^* = 0.1, 0.5$, and 0.9 (first, second, and third set of rows respectively).

K^*	$N = 2$	$N = 3$	$N = 4$	$N = \infty$
1	1.477	1.401	1.366	1.271
	6.770	7.138	7.321	7.868
0.1	1.487	1.365	1.315	0.968
	6.725	7.326	7.605	10.331
0.01	1.020	1.000	0.991	0.951
	9.804	10.000	10.091	10.515
1	1.477	1.401	1.366	1.271
	6.770	7.138	7.321	7.868
0.1	0.610	0.601	0.597	0.586
	16.393	16.639	16.750	17.065
0.01	0.529	0.526	0.524	0.520
	18.904	19.011	19.084	19.231
10	1.298	1.273	1.261	1.226
	7.704	7.855	7.930	8.157
1	0.211	0.210	0.209	0.207
	47.393	47.619	47.847	48.309
0.1	0.112	0.112	0.111	0.111
	89.286	89.286	90.090	90.090
0.01	0.102	0.102	0.102	0.102
	98.039	98.039	98.039	98.039

for the ever-attainable conversion of substrate using a series of CSTR's¹²⁾.

The plug-flow reactor case is an idealized situation, and was included because it represents a limiting behavior. For this reactor configuration, the average and maximum percent errors are much larger than for a small finite number of reactors. When N is large, the percent deviation is a monotonically increasing function of K^* . In general, the deviation is larger for both N small or N large; the minimum occurs around 3 to 4 (see Tables 4 to 6). This result is very important because it permits one to conclude that for the most reasonable number of reactors in industrial terms the approximation tested yields minimum deviations in the design estimates.

It is interesting to note that, although deactivation of the enzyme brings about a dependence of the intermediate concentration ($C_{S,i,\text{opt}}^*$) on various parameters in addition to $C_{S,N}^*$, the dependence of $C_{S,i,\text{opt}}^*$ on parameter K^* is much stronger than on η . As expected, $Da_{\text{tot,opt}}$ decreases as K^* and η decrease and $C_{S,N}^*$ increases (cf. Tables 1 to 3).

Table 3. Values of $Da_{tot,opt}$ (first row) and $1/(\eta \cdot Da_{tot,opt})$ (second row) for various numbers of reactors and parameter K^* , assuming that $\eta = 1$, for $C_{S,N}^* = 0.1, 0.5$, and 0.9 (first, second, and third set of rows respectively).

K^*	$N = 2$	$N = 3$	$N = 4$	$N = \infty$
1	0.248	0.241	0.239	0.230
	4.032	4.149	4.184	4.348
0.1	0.121	0.120	0.119	0.117
	8.264	8.333	8.403	8.547
0.01	0.109	0.108	0.107	0.107
	9.174	9.259	9.346	9.346

Table 4. Percent deviations of the true $Da_{tot,opt}$ relative to those obtained under the assumption of negligible rate of deactivation ($1/(\eta \cdot Da_{tot,opt}) \gg 1$) for various numbers of reactors and values of parameter K^* , assuming that $\eta = 0.01$, for $C_{S,N}^* = 0.1, 0.5$, and 0.9 (first, second, and third set of rows respectively).

K^*	$N = 2$	$N = 3$	$N = 4$	$N = \infty$
1	2.16×10^{-3}	5.07×10^{-4}	3.70×10^{-3}	8.65×10^{-1}
0.1	8.03×10^{-4}	3.00×10^{-5}	1.93×10^{-4}	2.44×10^{-1}
0.01	2.17×10^{-3}	3.61×10^{-3}	4.07×10^{-3}	3.55×10^{-2}
10	1.19×10^{-3}	6.15×10^{-4}	1.54×10^{-3}	3.76×10^{-1}
1	1.37×10^{-3}	1.45×10^{-4}	1.04×10^{-3}	2.09×10^{-1}
0.1	2.46×10^{-5}	2.03×10^{-5}	6.74×10^{-5}	4.53×10^{-2}
0.01	8.68×10^{-4}	7.89×10^{-4}	6.29×10^{-4}	7.39×10^{-3}
10	2.00×10^{-3}	4.51×10^{-3}	1.21×10^{-3}	5.42×10^{-2}
1	1.08×10^{-3}	2.27×10^{-3}	6.30×10^{-4}	2.81×10^{-2}
0.1	2.54×10^{-4}	5.41×10^{-4}	1.17×10^{-4}	5.62×10^{-3}
0.01	4.31×10^{-4}	4.98×10^{-4}	4.50×10^{-6}	1.09×10^{-3}

As a final conclusion, the approximation described by Eq. (6) proves helpful in the preliminary design of an industrial cascade of CSTR's performing the Michaelis-Menten reaction in the presence of enzyme deactivation because (i) at this calculation level the average (and also the maximum) error of such an assumption is acceptable for tentative estimation of the final optimum reactor volume and (ii) the time required for calculation of the approximate $Da_{tot,opt}$ under the assumption of negligible deactivation is orders of magnitude smaller than that required for calculation of the true $Da_{tot,opt}$ (viz., ca. 1 sec and ca. 15 min, respectively, for $N = 4$, $\eta = 1$ and $K^* = 4$, using a common tabletop microcomputer).

Nomenclature

$C_{S,0}$	= concentration of substrate at the inlet stream of the first reactor	[mol·m ⁻³]
$C_{S,1}$	= concentration of substrate at the outlet stream of the i -th reactor	[mol·m ⁻³]
$C_{S,N}$	= concentration of substrate at the outlet stream of the N -th reactor	[mol·m ⁻³]

Table 5. Percent deviations of the true $Da_{tot,opt}$ relative to those obtained under the assumption of negligible rate of deactivation ($1/(\eta \cdot Da_{tot,opt}) \gg 1$) for various numbers of reactors and values of parameter K^* , assuming that $\eta = 0.1$, for $C_{S,N}^* = 0.1, 0.5$, and 0.9 (first, second, and third set of rows respectively).

K^*	$N = 2$	$N = 3$	$N = 4$	$N = \infty$
0.1	4.42×10^{-2}	5.14×10^{-2}	4.86×10^{-2}	3.25×10^{-1}
0.01	1.85×10^{-1}	2.19×10^{-1}	1.94×10^{-1}	9.59×10^{-2}
1	1.23×10^{-4}	2.06×10^{-4}	5.34×10^{-4}	2.79×10^{-1}
0.1	8.81×10^{-3}	7.68×10^{-3}	5.72×10^{-3}	7.36×10^{-2}
0.01	2.55×10^{-2}	2.05×10^{-2}	1.63×10^{-2}	3.17×10^{-2}
10	9.40×10^{-3}	5.27×10^{-3}	5.61×10^{-3}	1.12×10^{-1}
1	1.19×10^{-3}	3.36×10^{-3}	7.49×10^{-4}	3.77×10^{-2}
0.1	2.63×10^{-4}	1.10×10^{-3}	8.01×10^{-5}	1.06×10^{-2}
0.01	1.74×10^{-4}	1.10×10^{-3}	8.74×10^{-5}	5.65×10^{-3}

Table 6. Percent deviations of the true $Da_{tot,opt}$ relative to those obtained under the assumption of negligible rate of deactivation ($1/(\eta \cdot Da_{tot,opt}) \gg 1$) for various numbers of reactors and values of parameter K^* , assuming that $\eta = 1$, for $C_{S,N}^* = 0.1, 0.5$, and 0.9 (first, second, and third set of rows respectively).

K^*	$N = 2$	$N = 3$	$N = 4$	$N = \infty$
1	1.49×10^{-3}	1.89×10^{-3}	1.23×10^{-2}	1.46×10^{-1}
0.1	2.98×10^{-3}	4.90×10^{-3}	1.99×10^{-3}	6.39×10^{-2}
0.01	2.44×10^{-3}	4.10×10^{-3}	1.22×10^{-3}	5.39×10^{-2}

$C_{S,pi}^*$ = normalized counterpart of $C_{S,i}$ [-],

$$C_{S,i}^* \equiv \frac{C_{S,i}}{C_{S,0}}$$

Da_i = Damköhler number associated with the i -th reactor [-],

$$Da_i \equiv \frac{V_{max,0} V_i}{Q C_{S,0}}$$

Da_{tot} = overall Damköhler number [-],

$$Da_{tot} \equiv \sum_{i=1}^N Da_i$$

i = integer variable [-]

j = dummy integer variable [-]

k = dummy integer variable [-]

k_d = deactivation constant [s⁻¹]

K_m = Michaelis-Menten parameter [mol·m⁻³]

K^* = dimensionless counterpart of K_m [-]

$$K^* \equiv \frac{K_m}{C_{S,0}}$$

I = dummy integer variable [-]

N = number of reactors in the series [-]

Q = volumetric flow rate of reacting liquid through the reactor network [m³·s⁻¹]

V_{max} = rate under saturation conditions of substrate [mol·m⁻³·s⁻¹]

$v_{max,0}$	= counterpart of v_{max} in the absence of enzyme deactivation	[mol. m ⁻³ . s ⁻¹]
V_i	= volume of the i -th reactor	[m ³]
η	= normalized deactivation constant	[-],
$\eta \equiv \frac{K_d}{\left(\frac{V_{max,0}}{C_{S,0}} \right)}$		

<Subscripts>

opt	= referring to the conditions leading to a minimum in the overall reactor volume
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