OPTIMAL DESIGN OF A SERIES OF CSTR's PERFORMING REVERSIBLE REACTIONS CATALYZED BY SOLUBLE ENZYMES: A THEORETICAL STUDY

F. XAVIER MALCATA†

Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200 Porto, Portugal

and

DOUGLAS C. CAMERON

Department of Chemical Engineering, University of Wisconsin, 1415 Johnson Drive, Madison, WI 53706, USA

The condition for the minimum overall reactor volume of a given number of CSTR's in series is theoretically determined for a reversible, single reactant–single product (Uni–Uni) enzyme catalyzed reaction. The reactor network is assumed to operate in steady-state, isothermal conditions with a single phase and a constant activity of biocatalyst. The method is based on a mathematical analysis of the discrete substrate concentration profile along the CSTR's assuming complete micromixing. The algebraic equations describing the critical loci are obtained for the general case, the mathematical proof that these equations define a minimum is presented, and an exact solution arising from an asymptotic situation is found. An approximate analytical method of optimization based on the aforementioned critical behavior is reported and its validity and usefulness discussed. The formulae introduced can be used in more general situations as tools for getting the approximate range where the optimal overall volume of the series of CSTR's lies. Hence, the reasoning developed is important for the preliminary CSTR design and relevant in the initial steps of the more involved methods of numerical optimization. Finally, the enzymatic conversion of fumarate to L-malate is examined as a model system in order to assess the usefulness and applicability of the analysis developed.

KEY WORDS CSTR's in series, minimum overall reactor volume, single substrate reversible reaction, homogeneous enzymatic catalysis, steady state, fumarase.

INTRODUCTION

All reactions catalyzed by enzymes are inherently reversible. Although a major fraction of intracellular pathways are reversible, the approach to equilibrium seldom presents a real problem to the cell because normally the reaction product immediately becomes the reactant of the next reaction in the pathway (i.e., the equilibrium composition is shifted towards greater conversion extents according

† To whom correspondence should be addressed.

to Le Châtelier's principle). Due to technical constraints, segmentation of the natural metabolic pathways is required before trying to perform enzyme-catalyzed reactions *in vitro*; only a very limited number of steps will actually be carried out, so reversible reactions become more likely to be of importance. On the industrial level, however, approach to equilibrium plays an important role in very few reactions. This situation can be mainly attributed to economic factors that limit the commercial feasibility of enzyme-catalyzed reversible reactions not only to cases where extremely expensive enzymes and cofactors are not required, but also to cases where a desired product can be manufactured at much milder conditions, at much larger rates, and with a much higher degree of selectivity than using chemical process counterparts.

The most widespread enzymatic process involving a reversible reaction that approaches equilibrium at relatively low conversions of substrate is the industrial production of high fructose corn syrup from starch hydrolysates using glucose isomerase: this reaction has, nevertheless, been traditionally carried out using immobilized enzyme technology. Another industrial applications is the preparation of L-malic acid, a general purpose food ingredient (Gardner, 1968), from fumaric acid using free microbial fumarase (Irwin *et al.*, 1967). Enzyme-catalyzed hydration of fumarate offers advantages over the chemical means (Weizmann, 1937) in that (i) it produces only a single optically active isomer, the naturally occurring form (i.e., L-malic acid), and (ii) maximum conversion efficiency is obtained at mild operating conditions, where acids are not particularly corrosive to the reactor material or coating (Irwin *et al.*, 1967).

Although for enzyme-catalyzed homogeneous reactions a plug-flow reactor gives a volumetric reaction rate that is superior to a (single) CSTR of equal volume, the continuous stirred tank reactor possesses a number of advantages for industrial operation. These include (i) lower construction costs when compared to classical tubular reactors, (ii) efficient stirring of the reactor content yielding uniform temperature and composition throughout, and (iii) easy access to the interior surface for maintenance (Hill, 1977).

Continuous stirred tank reactors have been successfully employed on the industrial level to perform biochemical reactions whenever (i) large volumetric flow rates of raw materials are handled and the products thereby obtained are subject to strict specifications, (ii) the cost of enzyme makeup is not too high or the level of purity of the enzyme with respect to other inert species is not critical for efficient operation, and (iii) the residual enzymatic activity in the effluent stream can be easily destroyed via thermal treatment or otherwise. If the cost of the enzyme plays the role of an important economic constraint, immobilization of the enzyme has proved to be a practical solution. However, coupling the CSTR with ultrafiltration membranes located downstream aimed at separating the residual enzyme from low molecular products via enforced flow across the membrane, followed by recycle of the enzyme to the CSTR has been reported to be a successful alternative (Wandrey, 1979).

Several literature references are available on the theoretical optimization procedures used in the design of a series of CSTR's performing biochemical reactions. From the work by Luyben and Tramper (1982) who introduced an analytical method to find the minimum overall volume of the reactor network for the case of a soluble enzyme following irreversible Michaelis–Menten kinetics, a number of extensions have emerged. These include (i) the case of irreversible

bireactant enzymatic reactions using two sequential objective functions in order to determine an optimal configuration which gives the minimum capital investment for a given set of operating conditions (Malcata, 1988), (ii) the case of a reaction catalyzed by an allosteric enzyme possessing positive cooperativity (Malcata, 1989a), (iii) the case of immobilized biocatalyst beads obeying intrinsic Michaelis-Menten kinetics (Gooijer *et al.*, 1989), (iv) the use of equipment costs based on variable scale-up exponential factors on the volume for the optimal design of a cascade of CSTR's (Malcata, 1989b), and (v) the change in the optimization techniques for the CSTR size profile in order to include the limiting case of complete segregation (Malcata, 1989c).

This paper deals with the development of a mathematical analysis aimed at finding the optimal design of a series of CSTR's assuming a reversible, single-substrate, enzyme-catalyzed biochemical reaction taking place in a homogeneous phase. The objective function is set to minimize the overall reactor volume. The particular case of having equal Michaelis-Menten constants for the reactant and product is given special attention since it leads to a simple functional form for the loci of the optimum intermediate concentrations of substrate. This asymptotic case is used as a basis for an approximate method of solution. The advantages and limitations thereof are discussed. In addition, the aforementioned method may yield approximate bounds for the overall reactor volume. This range may be employed during the process of guessing starting estimates for the more general situations of CSTR network design. Finally a numerical example is worked out in order to outline the applicability and usefulness of the reported theoretical analysis.

MATHEMATICAL ANALYSIS

Strictly speaking, all enzyme-catalyzed reactions are reversible. A realistic sequence involves the formation of a hybrid enzyme/substrate complex, *ES*, according to the following elementary mechanism (Segel, 1975; Bailey and Ollis, 1986)

$$E + S \rightleftarrows ES \rightleftarrows E + P \tag{1}$$

where E, S, and P denote the enzyme, substrate, and product, respectively. The net rate of the enzymatic conversion of S to P under the usual assumption of $C_S \gg C_{enz}$ can be obtained using the following relationship derived by Haldane (1930):

$$v \equiv -\frac{dC_S}{dt} \equiv \frac{dC_P}{dt} = \frac{v_{max,f} K_P C_S - v_{max,r} K_S C_P}{K_S K_P + K_P C_S + K_S C_P} \tag{2}$$

It should be emphasized here that, due to the reversible nature of the reaction under study, v may be either positive or negative; however, for the sake of simplicity of the mathematical analysis, it will hereafter be assumed that S denotes the substance that is consumed and P denotes the substance that is formed by the enzyme-catalyzed reaction. At equilibrium, the net rate of the

biochemical reaction becomes nil. Equation (2) can then be transformed into

$$K_{eq} \equiv \frac{C_{P,eq}}{C_{S,eq}} = \frac{v_{max,f} K_P}{v_{max,r} K_S} \tag{3}$$

Consider, as a working example, a system of CSTR's in series performing a homogeneous, enzyme-catalyzed reaction (such as the conversion of fumarate to L-malate) in steady state, with the reaction kinetics being described by Equation (2). The enzyme concentration in all reactors is assumed to be equal and remain constant at all times. (If the half life of the enzyme is very large compared with the overall space time associated with the cascade of reactors, the condition of equal and constant enzyme concentration in all reactors is fulfilled provided that fresh, or recycled, enzyme is added to the inlet stream of the first reactor in amounts that make up for the enzyme lost in the outlet stream of the last reactor.) Isothermal conditions of operation are assumed. There are no side reactions taking place and the reaction starts with an aqueous solution of both reactant and product. It is intended to design the volume of each unit in the series of CSTR's in such a way that, for a given overall conversion of substrate, the overall reactor volume is minimized.

The stoichiometry of the reaction allows one to obtain the following expression relating the concentrations of reactant and product:

$$C_{S,0} + C_{P,0} = C_{S,i} + C_{P,i} \tag{4}$$

The material balance on the reactant in each CSTR of the series can be expressed as

$$Q(C_{S,i-1} - C_{S,i}) = V_i \frac{v_{max,f}\left(\frac{C_{S,i}}{K_S}\right) - v_{max,r}\left(\frac{C_{P,i}}{K_P}\right)}{1 + \left(\frac{C_{S,i}}{K_S}\right) + \left(\frac{C_{P,i}}{K_P}\right)}, \quad i = 1, 2, \ldots, N \tag{5}$$

Use of Equation (4) in Equation (5) and performance of some algebraic manipulation on the result thereby obtained leads to the following equation

$$\mathrm{Da}_i = \frac{K_{eq}(C^*_{S,i-1} - C^*_{S,i})((K^*_P - K^*_S)C^*_{S,i} + K^*_S(1 + M + K^*_P))}{K^*_P((1 + K_{eq})C^*_{S,i} - (1 + M))}, \quad i = 1, 2, \ldots, N \tag{6}$$

Equation (4) can be rearranged to read

$$1 + M = C^*_{S,i} + C^*_{P,i} \tag{7}$$

In the above equations, the ratio of product to reactant initial concentrations was denoted as M. The Damköhler number can be ascribed the following meaning: Da_i is the ratio of the space time, or average residence time of reactor i(i.e., V_i/Q) to the characteristic time scale associated with the chemical reaction if it were assumed to be irreversible and follow zero order kinetics at all times (i.e., $C_{S,0}/v_{max,f}$). The definition of this number is convenient because it helps in removing overparameterization from the problem and allows the analysis to be pursued in dimensionless form. In a sense, Da_i can be viewed as the dimensionless volume of the i-th reactor.

A necessary mathematical condition for a critical point reads (Luyben and Tramper, 1982)

$$\left\{\frac{\partial \mathrm{Da}_{\mathrm{tot}}}{\partial C^*_{S,i}}\right\}_{K_{\mathrm{eq}},K^*_P,K^*_S,M,C^*_{S,-i}} \equiv \frac{\partial}{\partial C^*_{S,i}}\left\{\sum_{j=1}^{N} \mathrm{Da}_j\right\}_{K_{\mathrm{eq}},K^*_P,K^*_S,M,C^*_{S,-i}} = 0, \quad i = 1, 2, \ldots, N-1 \tag{8}$$

where $C^*_{S,-i}$ denotes all values of $C^*_{S,j}$ ($j = 1, 2, \ldots, i-1, i+1, \ldots, N$). Since $C^*_{S,i}$ appears only in the i-th and the $(i+1)$-th terms of the foregoing summation, Equation (8) is equivalent to

$$\left\{\frac{\partial \mathrm{Da}_i}{\partial C^*_{S,i}}\right\}_{K_{\mathrm{eq}},K^*_P,K^*_S,M,C^*_{S,-i}} + \left\{\frac{\partial \mathrm{Da}_{i+1}}{\partial C^*_{S,i}}\right\}_{K_{\mathrm{eq}},K^*_P,K^*_S,M,C^*_{S,-i}} = 0, \quad i = 1, 2, \ldots, N-1 \tag{9}$$

Combining Equations (6) and (9) one obtains

$$\frac{K_{\mathrm{eq}}((C^*_{S,i-1,\mathrm{opt}} - C^*_{S,i,\mathrm{opt}})(K^*_P - K^*_S) - ((K^*_P - K^*_S)C^*_{S,i,\mathrm{opt}} + K^*_S(1 + M + K^*_P)))}{K^*_P((1 + K_{\mathrm{eq}})C^*_{S,i,\mathrm{opt}} - (1 + M))}$$

$$- \frac{K_{\mathrm{eq}}K^*_P(1 + K_{\mathrm{eq}})(C^*_{S,i-1,\mathrm{opt}} - C^*_{S,i,\mathrm{opt}})((K^*_P - K^*_S)C^*_{S,i,\mathrm{opt}} + K^*_S(1 + M + K^*_P))}{(K^*_P((1 + K_{\mathrm{eq}})C^*_{S,i,\mathrm{opt}} - (1 + M)))^2}$$

$$+ \frac{K_{\mathrm{eq}}((K^*_P - K^*_S)C^*_{S,i+1,\mathrm{opt}} + K^*_S(1 + M + K^*_P))}{K^*_P((1 + K_{\mathrm{eq}})C^*_{S,i+1,\mathrm{opt}} - (1 + M))} = 0, \quad i = 1, 2, \ldots, N-1 \tag{10}$$

Algebraic manipulation of Equation (10) yields

$$\frac{(K^*_P - K^*_S)C^*_{S,i+1,\mathrm{opt}} + K^*_S(1 + M + K^*_P)}{(1 + K_{\mathrm{eq}})C^*_{S,i+1,\mathrm{opt}} - (1 + M)}$$

$$- \frac{(K^*_P - K^*_S)C^*_{S,i,\mathrm{opt}} + K^*_S(1 + M + K^*_P) - (C^*_{S,i-1,\mathrm{opt}} - C^*_{S,i,\mathrm{opt}})(K^*_P - K^*_S)}{(1 + K_{\mathrm{eq}})C^*_{S,i,\mathrm{opt}} - (1 + M)}$$

$$= \frac{(1 + K_{\mathrm{eq}})(C^*_{S,i-1,\mathrm{opt}} - C^*_{S,i,\mathrm{opt}})((K^*_P - K^*_S)C^*_{S,i,\mathrm{opt}} + K^*_S(1 + M + K^*_P))}{((1 + K_{\mathrm{eq}})C^*_{S,i,\mathrm{opt}} - (1 + M))^2}, \quad i = 1, 2, \ldots, N-1 \tag{11}$$

Since the condition expressed by Equation (8) holds for a minimum, as well as for a maximum or an inflexion point, then the sign of the second order derivative at the critical point must be investigated. Recalling Equation (10), the second derivative of $\mathrm{Da}_{\mathrm{tot}}$ with respect to $C^*_{S,i}$ can be obtained as follows

$$\left\{\frac{\partial^2 \mathrm{Da}_{\mathrm{tot}}}{\partial C^{*2}_{S,i}}\right\}_{K_{\mathrm{eq}},K^*_P,K^*_S,M,C^*_{S,-i}} = \left\{\frac{\partial}{\partial C^*_{S,i}}\left\{\frac{\partial \mathrm{Da}_i}{\partial C^*_{S,i}}\right\}_{K_{\mathrm{eq}},K^*_P,K^*_S,M,C^*_{S,-i}}\right\}_{K_{\mathrm{eq}},K^*_P,K^*_S,M,C^*_{S,-i}} =$$

$$- \frac{K_{\mathrm{eq}}(K^*_P - K^*_S)}{K_P((1 + K_{\mathrm{eq}})C^*_{S,i} - (1 + M))} - \frac{K_{\mathrm{eq}}K^*_P(K^*_P - K^*_S)(1 + K_{\mathrm{eq}})(C^*_{S,i-1} - C^*_{S,i})}{(K^*_P((1 + K_{\mathrm{eq}})C^*_{S,i} - (1 + M)))^2}$$

$$- \frac{K_{\mathrm{eq}}(K^*_P - K^*_S)}{K_P((1 + K_{\mathrm{eq}})C^*_{S,i} - (1 + M))} + \frac{K_{\mathrm{eq}}K^*_P(1 + K_{\mathrm{eq}})((K^*_P - K^*_S)C^*_{S,i} + K^*_S(1 + M + K^*_P))}{(K^*_P(1 + K_{\mathrm{eq}})C_{S,i} - (1 + M)))^2}$$

$$-\frac{K_{eq}K_P^*(1+K_{eq})((K_P^*-K_S^*)(C_{S,i-1}^*-C_{S,i}^*)-((K_P^*-K_S^*)C_{S,i}^*+K_S^*(1+M+K_P^*)))}{(K_P^*((1+K_{eq})C_{S,i}^*-(1+M)))^2}$$

$$+\frac{2K_{eq}K_P^{*2}(1+K_{eq})^2(C_{S,i-1}^*-C_{S,i}^*)((K_P^*-K_S^*)C_{S,i}^*+K_S^*(1+M+K_P^*))}{(K_P^*((1+K_{eq})C_{S,i}^*-(1+M)))^3},$$

$$i=1,2,\ldots,N-1 \quad (12)$$

where advantage was taken from the fact that $\{\partial Da_{i+1}/\partial C_{S,i}^*\}_{K_{eq},K_P^*,K_S^*,M,C_{S,i-1}^*}$ is not a function of $C_{S,i}^*$. Grouping similar terms and factoring out common terms in Equation (12), one obtains

$$-\frac{K_P^*}{2K_{eq}}\left\{\frac{\partial^2 Da_{tot}}{\partial C_{S,i}^{*2}}\right\}_{K_{eq},K_P^*,K_S^*,M,C_{S,i-1}^*}=\frac{K_P^*-K_S^*}{(1+K_{eq})C_{S,i}^*-(1+M)}$$

$$-\frac{(1+K_{eq})((K_P^*-K_S^*)(2C_{S,i}^*-C_{S,i-1}^*)+K_S^*(1+M+K_P^*))}{((1+K_{eq})C_{S,i}^*-(1+M))^2}$$

$$-\frac{(1+K_{eq})^2(C_{S,i-1}^*-C_{S,i}^*)((K_P^*-K_S^*)C_{S,i}^*+K_S^*(1+M+K_P^*))}{((1+K_{eq})C_{S,i}^*-(1+M))^3},$$

$$i=1,2,\ldots,N-1 \quad (13)$$

Reducing the terms of the RHS of Equation (13) to the same denominator, performing the multiplications thus obtained, grouping similar terms, and factoring out common terms, Equation (13) can be rearranged to read

$$\left\{\frac{\partial^2 Da_{tot}}{\partial C_{S,i}^{*2}}\right\}_{K_{eq},K_P^*,K_S^*,M,C_{S,i-1}^*}=$$

$$\frac{2K_{eq}((1+K_{eq})C_{S,i-1}^*-(1+M))(1+M)(K_P^*-K_S^*)+K_S^*(1+K_{eq})(1+M+K_P^*))}{K_P^*((1+K_{eq})C_{S,i}^*-(1+M))^3} \quad (14)$$

Since the Damköhler numbers are continuous functions of the intermediate substrate concentrations, Equation (8) represents a necessary, but not a sufficient, conditions for a local minimum of Da_{tot}. Besides setting the first derivatives equal to zero, one must confirm that the second order derivatives with respect to each $C_{S,i}^*$ are positive. This fact is apparent from a careful inspection of the sign of the right hand side of Equation (14): the differences appearing in the numerator and in the denominator must be positive in order to ensure physical consistency (i.e., if the reaction system evolves towards a thermodynamic equilibrium, such differences must remain positive).

Returning to Equation (11), an analytical solution does not exist for it in the general form. Therefore, one must resort to numerical procedures to obtain $C_{S,i,opt}^*$. In order for these procedures to be computationally efficient, good initial estimates are required. An approximate range for these estimates can be easily obtained from consideration of the asymptotic situation of having K_S^* and K_P^* with the same value. Using this mathematical convenience, the terms containing $(K_P^*-K_S^*)$ in Equation (11) can be dropped, and thus considerable simplification of this equation is obtained, viz.

$$(C_{S,i,opt,ap}^*(1+K_{eq})-(1+M))^2$$
$$=(C_{S,i-1,opt,ap}^*(1+K_{eq})-(1+M))(C_{S,i+1,opt,ap}^*(1+K_{eq})-(1+M)) \quad (15)$$

Table 1 Values of $(C^*_{S,i,\mathrm{opt,ap}} - C^*_{S,\mathrm{eq}})/(1 - C^*_{S,\mathrm{eq}})$ at the outlet of each reactor assuming that $(C^*_{S,N} - C^*_{S,\mathrm{eq}})/(1 - C^*_{S,\mathrm{eq}}) = 0.100$ and $K^*_S = K^*_P$ (first eleven columns), followed by the corresponding Damköhler number normalized by the Damköhler number for a plug flow reactor (last column).

N	i	0	1	2	3	4	5	6	7	8	9	10	$Da_{tot,opt,ap}/Da_{pf}$
1		1.000	0.100										3.909
2		1.000	0.316	0.100									1.878
3		1.000	0.464	0.215	0.100								1.504
4		1.000	0.562	0.316	0.178	0.100							1.352
5		1.000	0.631	0.398	0.251	0.158	0.100						1.270
6		1.000	0.681	0.464	0.316	0.215	0.147	0.100					1.219
7		1.000	0.720	0.518	0.373	0.268	0.193	0.139	0.100				1.184
8		1.000	0.750	0.562	0.422	0.316	0.237	0.178	0.133	0.100			1.159
9		1.000	0.774	0.599	0.464	0.359	0.278	0.215	0.167	0.129	0.100		1.140
10		1.000	0.794	0.631	0.501	0.398	0.316	0.251	0.200	0.158	0.126	0.100	1.124

Applying the recursive relation denoted as Equation (15) from $i = 1$ up to $i = N$, one gets after rearrangement

$$\frac{(1 + K_{\mathrm{eq}})C^*_{S,i,\mathrm{opt,ap}} - (1 + M)}{K_{\mathrm{eq}} - M} = \left(\frac{(1 + K_{\mathrm{eq}})C^*_{S,N} - (1 + M)}{K_{\mathrm{eq}} - M}\right)^{i/N} \tag{16}$$

Combining Equations (6) and (16), one finally obtains

$$\mathrm{Da}_{i,\mathrm{opt,ap}} = \frac{K^*_S K_{\mathrm{eq}}(1 + M + K^*_P)}{K^*_P(1 + K_{\mathrm{eq}})}\left(\left(\frac{K_{\mathrm{eq}} - M}{(1 + K_{\mathrm{eq}})C^*_{S,N} - (1 + M)}\right)^{1/N} - 1\right) \tag{17}$$

The optimal values of the intermediate concentrations for three different conversions of reactant are found in Tables 1, 2, and 3. Included are also the corresponding overall Damköhler numbers normalized by the Damköhler number of an equivalent plug flow reactor. Special advantage was taken from the fact that $[(1 + K_{\mathrm{eq}})C^*_{S,i} - (1 + M)]/(K_{\mathrm{eq}} - M)$ is formally equivalent to $(C^*_{S,i} - C^*_{S,\mathrm{eq}})/(1 - C_{S,\mathrm{eq}})$ in order to enhance the physical significance of the former expression as the degree of approach to equilibrium conditions.

As the number of reactors in series increases, the total Dahköhler number

Table 2 Values of $(C^*_{S,i,\mathrm{opt,ap}} - C^*_{S,\mathrm{eq}})/(1 - C^*_{S,\mathrm{eq}})$ at the outlet of each reactor assuming that $(C^*_{S,N} - C^*_{S,\mathrm{eq}})/(1 - C^*_{S,\mathrm{eq}}) = 0.500$ and $K^*_S = K^*_P$ (first eleven columns), followed by the corresponding Damköhler number normalized by the Damköhler number for a plug flow reactor (last column).

N	i	0	1	2	3	4	5	6	7	8	9	10	$Da_{tot,opt,ap}/Da_{pf}$
1		1.000	0.500										1.443
2		1.000	0.707	0.500									1.195
3		1.000	0.794	0.630	0.500								1.125
4		1.000	0.841	0.707	0.595	0.500							1.092
5		1.000	0.871	0.758	0.660	0.574	0.500						1.073
6		1.000	0.891	0.794	0.707	0.630	0.561	0.500					1.060
7		1.000	0.906	0.820	0.743	0.673	0.610	0.552	0.500				1.051
8		1.000	0.917	0.841	0.771	0.707	0.648	0.595	0.545	0.500			1.045
9		1.000	0.926	0.857	0.794	0.735	0.680	0.630	0.583	0.540	0.500		1.040
10		1.000	0.933	0.871	0.812	0.758	0.707	0.660	0.616	0.574	0.536	0.500	1.035

Table 3 Values of $(C^*_{S,i,\text{opt,ap}} - C^*_{S,\text{eq}})/(1 - C^*_{S,\text{eq}})$ at the outlet of each reactor assuming that $(C^*_{S,N} - C^*_{S,\text{eq}})/(1 - C^*_{S,\text{eq}}) = 0.900$ and $K^*_S = K^*_P$ (first eleven columns), followed by the corresponding Damköhler number normalized by the Damköhler number for a plug flow reactor (last column).

N	i 0	1	2	3	4	5	6	7	8	9	10	$Da_{tot,opt,ap}/Da_{pf}$
1	1.000	0.900										1.055
2	1.000	0.949	0.900									1.027
3	1.000	0.965	0.932	0.900								1.018
4	1.000	0.974	0.949	0.924	0.900							1.013
5	1.000	0.979	0.958	0.939	0.919	0.900						1.011
6	1.000	0.983	0.965	0.949	0.932	0.916	0.900					1.009
7	1.000	0.985	0.970	0.956	0.942	0.928	0.914	0.900				1.008
8	1.000	0.987	0.974	0.961	0.949	0.936	0.924	0.912	0.900			1.007
9	1.000	0.988	0.977	0.965	0.954	0.943	0.932	0.921	0.911	0.900		1.006
10	1.000	0.990	0.979	0.969	0.959	0.949	0.939	0.929	0.919	0.910	0.900	1.005

tends towards a limit according to Equation (18):

$$\lim_{N\to\infty} \text{Da}_{\text{tot,opt,ap}} = \text{Da}_{\text{pf}} = \frac{K^*_S K_{\text{eq}}(1 + M + K^*_P)}{K^*_P(1 + K_{\text{eq}})} \ln\left\{\frac{K_{\text{eq}} - M}{(1 + K_{\text{eq}})C^*_{S,N} - (1 + M)}\right\} \tag{18}$$

where the total Damköhler number is given by

$$\text{Da}_{\text{tot,opt,ap}} \equiv \sum_{j=1}^{N} \text{Da}_{j,\text{opt,ap}} = N\,\text{Da}_{i,\text{opt,ap}}, \quad i = 1, 2, \ldots, N \tag{19}$$

The value of Da_{pf} can also be obtained from the material balance to a plug flow reactor performing the same reaction (Spiegel, 1968)

$$\int_0^{\text{Da}_{\text{pf}}} d\zeta + \frac{K_{\text{eq}}}{K^*_P} \int_1^{\xi_N} \frac{(K^*_P - K^*_S)\xi + K^*_S(1 + M + K^*_P)}{(1 + K_{\text{eq}})\xi - (1 + M)} d\xi = 0 \tag{20}$$

where Equation (7) was again used to eliminate the functional dependence on $C^*_{P,i}$. The fact that the plug flow reactor provides a boundary for the total volume of the reactor is apparent from previous work (Luyben and Tramper, 1982; Malcata, 1988).

Although exact only for $K^*_S = K^*_P$, Equation (17) can also be employed as a useful approximation for cases where this equality condition is only approached provided that the calculation of an estimate of the error implicit in such an approximation is feasible. From the definition of dimensionless substrate concentration and the assumption that the reaction in the forward direction is faster than in the reverse direction (i.e., $C_{P,0}/C_{S,0} < K_{\text{eq}}$), it is obvious that $C^*_{S,i} \leq 1$ for $i = 1, 2, \ldots, N$; this implies that $(K^*_P - K^*_S)C^*_{S,i} + K^*_S(1 + M + K^*_P) \leq (K^*_P - K^*_S) + K^*_S(1 + M + K^*_P)$. Careful inspection of the form of the numerator of Equation (6) allows one to find that, given $C^*_{S,i-1}$ and $C^*_{S,i}$, an upper bound for Da_i is then obtained when $(K^*_P - K^*_S)C^*_{S,i} + K^*_S(1 + M + K^*_P) \sim K^*_P(1 + K^*_S) + MK^*_S$. A reasoning similar to the one that led to the derivation of Equation (16) from Equations (6) and (9)–(11) can now be followed for this hypothetical situation thus enabling one to obtain the optimal intermediate concentrations as expressed by the following equation:

$$\frac{(1 + K_{\text{eq}})C^*_{S,i,\text{opt,est}} - (1 + M)}{K_{\text{eq}} - M} = \left(\frac{(1 + K_{\text{eq}})C^*_{S,N} - (1 + M)}{K_{\text{eq}} - M}\right)^{i/N} \tag{21}$$

Using Equation (21) in the corresponding simplified form of Equation (6), one finds that

$$\mathrm{Da}_{i,\mathrm{opt,est}} = \frac{K_S^* K_{\mathrm{eq}} \left(\frac{K_P^*}{K_S^*} + M + K_P^* \right)}{K_P^* (1 + K_{\mathrm{eq}})} \left(\left(\frac{K_{\mathrm{eq}} - M}{(1 + K_{\mathrm{eq}}) C_{S,N}^* - (1 + M)} \right)^{1/N} - 1 \right) \quad (22)$$

For cases where the conversion of S remains low (say, below 10%), a good estimate of the relative deviation implicit in assuming that K_S^* equals K_P^* when this is only approximately true then reads

$$Er \equiv \frac{\mathrm{Da}_{i,\mathrm{opt,ap}} - \mathrm{Da}_{i,\mathrm{opt,est}}}{\mathrm{Da}_{i,\mathrm{opt,est}}} = \frac{K_S^* - K_P^*}{K_P^* + K_S^* (M + K_P^*)} \quad (23)$$

irrespective of the actual values of N, K_{eq}, and $C_{S,N}^*$. The values for Er (which are independent of the position of the reactor in the cascade of CSTR's) are estimates of the actual deviation defined as

$$Dv_i \equiv \frac{\mathrm{Da}_{i,\mathrm{opt,ap}} - \mathrm{Da}_{i,\mathrm{opt}}}{\mathrm{Da}_{i,\mathrm{opt}}} \quad (24)$$

The confidence contours on the K_S^*:K_P^* plane for a number of estimated percentual deviation levels are plotted in Figures 1(i) & (ii) for two arbitrary values of the ratio M. Two asymptotic values exist for $|Er|$, which play an important role at large K_S^* or K_P^*. These can be obtained from Equation (23) to be

$$\lim_{K_S^* \to \infty} |Er| = \frac{1}{M + K_P^*} \quad (25)$$

and

$$\lim_{K_P^* \to \infty} |Er| = \frac{1}{1 + K_S^*} \quad (26)$$

These limiting situations correspond to negligible resistance to the kinetic rate for the reverse and the forward reaction, respectively, arising from the binding of reactant or product.

In the general case of having $K_S^* \neq K_P^*$ and $0 < C_{S,N}^* \ll 1$ the exact value of $\mathrm{Da}_{\mathrm{tot,opt}}$ is contained in either the interval $[\mathrm{Da}_{\mathrm{tot,opt,ap}}, \mathrm{Da}_{\mathrm{tot,opt,est}}]$ or the interval $[\mathrm{Da}_{\mathrm{tot,opt,est}}, \mathrm{Da}_{\mathrm{tot,opt,ap}}]$ depending on whether $K_P > K_S$ or $K_P < K_S$, respectively. This result is emphasized in the following application.

NUMERICAL EXAMPLE

Consider the isothermal conversion of fumarate to L-malate at 25°C in a sodium phosphate aqueous solution of fumarase (EC 4.2.1.2) buffered at pH 7.0. The Michaelis constants have been found to be (Frieden *et al.*, 1957): $K_S = 7.2 \times 10^{-2}\ \mathrm{mol \cdot m^{-3}}$, and $K_P = 1.9 \times 10^{-1}\ \mathrm{mol \cdot m^{-3}}$. The turnover numbers associated with the forward and reverse reactions are $1.9 \times 10^3\ \mathrm{s^{-1}}$ and $1.1 \times 10^3\ \mathrm{s^{-1}}$, respectively (Frieden *et al.*, 1957); coupling of this value with the expected concentration of enzyme to be maintained in each reactor is (i.e., $5.0 \times$

10^{-7} mol · m^{-3}) gives $v_{max,f} = 9.5 \times 10^{-4}$ mol · m^{-3} · s^{-1} and $v_{max,r} = 5.5 \times 10^{-4}$ mol · m^{-3} · s^{-1}. Two CSTR's in series will be used to perform the aforementioned reaction with initial fumarate and L-malate concentrations of 35 mol · m^{-3} and 0.5 mol · m^{-3}, respectively. The available pumping capacity allows a volumetric flow rate of 4.85×10^{-5} $m^3 \cdot s^{-1}$ to be delivered. Separation of the unreacted substrate from the product in the effluent stream will be achieved by filtration upon cooling, advantage being taken from the much greater solubility of malic acid over fumaric acid in water (Dziezak, 1990). The residual enzymatic activity in the effluent stream will be destroyed by thermal treatment. Determine the volume of each reactor of the series yielding minimum global volume for the reactor network on the assumption that a 45% conversion of fumarate is desired.

Using Equation (3) coupled with the definitions of the kinetic parameters and the normalized concentrations as given in the Nomenclature, one obtains $K_S^* = 2.06 \times 10^{-3}$, $K_P^* = 5.43 \times 10^{-3}$, $K_{eq} = 4.56$, $M = 1.43 \times 10^{-2}$, and $C_{S,2}^* = 5.50 \times 10^{-1}$. Using these values in Equation (11) and performing some algebra, one obtains the following simplified expression

$$C_{S,1,opt}^{*2} - 0.5316 C_{S,1,opt}^* - 0.08596 = 0 \tag{27}$$

Of the two solutions of the polynomial that can be analytically obtained, only $C_{S,1,opt}^* = 0.6615$ is physically meaningful (the other solution is a negative value). Replacing this value in Equation (6), one finds that $Da_{1,opt} = 0.4620$ and $Da_{2,opt} = 0.1811$. Using the definitions of the Damköhler number as listed in the Nomenclature together with the remaining data available, one finally obtains that $V_{1,opt} = 0.8255$ m^3 and $V_{2,opt} = 0.3236$ m^3. If the assumption that $K_S^* \sim K_P^*$ were true then Equation (17) would yield equal values for $Da_{1,opt,ap}$ and $Da_{2,opt,ap}$ (i.e., 0.1557), and $V_{1,opt,ap} = V_{2,opt,ap} = 0.2782$ m^3. Recalling Equation (22), one would have obtained a common value for $Da_{1,opt,est}$ and $Da_{2,opt,est}$ (i.e., 0.4060), and thus $V_{1,opt,est} = V_{2,opt,est} = 0.7255$ m^3. The actual value for the minimum total value required to effect the aforementioned conversion (i.e., 1.1491 m^3) is between the total volume predicted by using Equation (17) (i.e., 0.5564 m^3) and the total volume obtained by using Equation (22) (i.e., 1.2510 m^3).

DISCUSSION

Under the usual assay conditions the velocities of reaction are measured very early in the reaction before the product concentration has increased to a significant level. In this situation Equation (2) reduces to the classical form of the Michaelis-Menten kinetic equation (Segel, 1975), thus allowing the kinetic constants for both forward and reverse reactions to be estimated from classical linear fits to initial velocity data (e.g., Lineweaver-Burke plots). The four lumped kinetic parameters are not, however, all independent due to the general principle of microreversibility (Hill, 1977). In fact, the relationship denoted as Equation (3), and commonly known as the Haldane equation (Creighton, 1984), can be understood as a thermodynamic constraint upon the biochemical reaction which depends only on the temperature of operation. Although the correct definition of the equilibrium constant (according to the general principles of mass action) requires the use of activities of the reactants and products instead of their molar concentrations, the aqueous solutions were assumed ideal (i.e., activity coefficient

equal to one) and dilute (i.e., molar fraction approximately equal to the solute-free molar fraction) for the sake of mathematical simplicity. The deviations from ideality and dilute behavior are not expected to introduce severe errors in common industrial practice.

It might be argued that the generic process scenario of having initial finite concentrations of both S and P (i.e., $M \neq 0$) is not reasonable from an economic point of view for an equilibrium limited conversion of S to P. The presence of some product of the forward reaction was considered in order to include situations where the natural feedstocks already contain both S and P rather than situations where some effluent stream was to be recycled to the first reactor in the cascade of CSTR's.

Careful inspection of Tables 1 through 3 allows one to find that the volume of a plug flow reactor becomes a better approximation of the total volume of the cascade of CSTR's as $(C^*_{S,N} - C^*_{S,eq})/(1 - C^*_{S,eq})$ increases. Moreover, the aforementioned approximation improves as N increases for each level of $(C^*_{S,N} - C^*_{S,eq})/(1 - C^*_{S,eq})$, as expected. In general, a preliminary estimate of the minimum size of the cascade of CSTR's may proceed via Equation (18) for N above, say, 6.

It is interesting to note that in the asymptotic case leading to Equation (16) the optimal intermediate substrate concentrations are a function of the equilibrium constant, the initial ratio of concentrations of reactant and product, the number of reactors, and the desired final concentration of substrate. Furthermore, the Damköhler number for each of the CSTR's in the series under the assumption of $K^*_S = K^*_P$, as expressed in Equation (17), for each set of parameters K_{eq}, K^*_S, K^*_P, M, and N is the same. The above finding is remarkable for the particular cases where K_S is close to K_P because it leads to equal-sized reactors, with corresponding ease of construction and stocking of spare parts. In the general case of applicability of Equation (11), it is found that individual reactors become progressively (with increasing i) smaller, as was already observed by Luyben and Tramper (1982) for the *irreversible* Michaelis-Menten counterpart.

The result previously reported by Luyben and Tramper (1982) for the case of an irreversible, single substrate enzymatic reaction following simple Michaelis-Menten kinetics can be obtained from Equation (11) as a limiting case. This particular solution requires that not only the chemical reaction proceeds virtually to completion (e.g., large K_{eq}), but also that the product generated in the forward reaction does not appreciably bind to the enzyme (i.e., large K^*_P). In this limiting case the loci of the intermediate concentration optima depend only on the number of reactors and the final desired conversion. If these assumptions were taken as valid with the data used in the above numerical example, then one would have gotten $C^*_{S,1,opt} = 0.7416$, $Da_{1,opt} = 0.2591$ and $Da_{2,opt} = 0.1923$; these values yield an underestimated minimum reactor size required for the expected conversion. This drawback increases rapidly in importance as the desired conversion approaches the equilibrium conversion.

The approximation suggested as the underlying rationale of Equations (23)–(24) [i.e., Equation (22)] leads in general to predicted errors (Er) of the order of magnitude of, but not necessarily greater than, the actual value of each Dv_i. This finding is due to the fact that although $C^*_{S,i}$ has an upper limit of unity, the structure of the optimization procedure as depicted in Equation (9) is distorted when one assumes that $C^*_{S,i} = 1$ *only* in the third term of the numerator of

Equation (6) [$C^*_{S,i}$ appears explicitely three times in Equation (6)]. The estimate Er increases in accuracy as the overall reactant conversion in the reactor network decreases because $C^*_{S,i}$ then remains closer to unity for all $i = 1, 2, \ldots, N$. In general, it can be stated that $|Er|$ is an upper bound for the average value of $|Dv_i|$ extending to all N reactors in the series (i.e., $\sum_{i=1}^{N} |Dv_i| \leq N \cdot |Er|$).

Data available for 1986 estimate the world overall consumption of malic acid as 20,000 ton/year (Blenford, 1986). Clear tendencies for increase of this figure in the near future are anticipated based on consumer trends (Blenford, 1986) and as a result of greater availability (Irwin *et al.*, 1967). A survey over some common suppliers of fine chemicals indicates that 1 kg of food grade fumaric acid and L-malic acid (both in free crystalline form) costs around 1 and 16 arbitrary currency units (c.u.), respectively. On the other hand, microbial raw fumarase costs about 0.00028 c.u./f.u., where the fumarase unit (f.u.) is defined as the amount of enzyme able to catalyze the production of 1 $\mu mol_{L\text{-malate}}$/min. The raw net profit is therefore 4.9 c.u./$kg_{L\text{-malate}}$ under the assumptions that (i) the

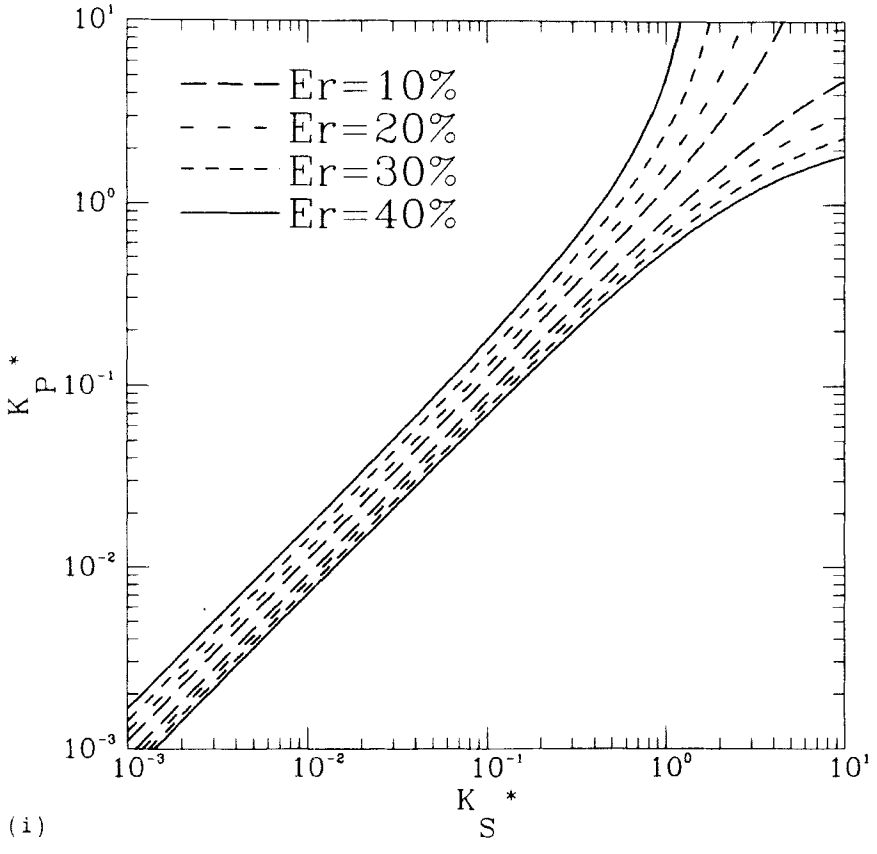

Figure 1 Confidence contours for a number of estimated percentual error levels, $|Er|$, plotted as K^*_P vs. K^*_S within the range of industrial interest. Two arbitrary values were considered for the initial ratio of product to reactant concentrations: $M = 0$ (part i) and $M = 1$ (part ii). The main diagonal corresponds to $Er = 0\%$.

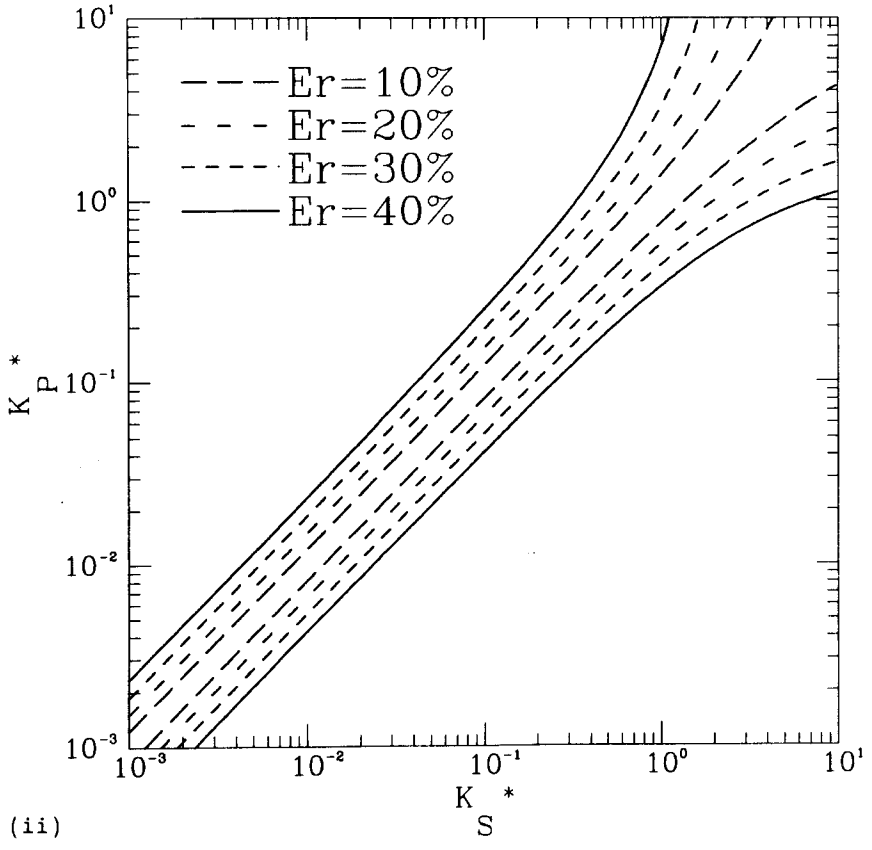

Figure 1 (continued)

residence time of the fluid is 10 h, (ii) 300 units of fumarase are used in the reactor system, (iii) all enzyme is lost upon one passage through the cascade of CSTR's, and (iv) the reaction rate is on average one half the maximum value. The reversible reaction catalyzed by fumarase in a homogeneous aqueous sytem is, therefore, of commercial interest and thus particularly suitable for design purposes.

The reaction considered in the problem statement involves two substrates in the forward reaction, one of which is water. The concentration of water remains, however, essentially constant because water also plays the role of solvent: the water concentration is typically two to three orders of magnitude greater than that of the substrate or product. In such situations the reaction can be virtually taken as unireactant in both directions and the reasoning followed remains valid.

The exact calculation of the optimal intermediate concentrations leading to the minimum overall reactor volume, although conceptually straightforward, is quite cumbersome in practice whenever a fairly large number of reactors is considered (for a cascade of N CSTR's, a set of $N-1$ N-th degree polynomials must be solved simultaneously). This often requires involved mathematical manipulations

which are more likely to lead to numerical instability as the number (and order) of the polynomials resulting from Equation (11) increases. The possible numerical solutions also increase in number, so special care is to be exercised in selecting the correct one. This can be achieved at the expense of appropriate physical constraints, which can be stated as $1 \geq C^*_{S,i-1,\mathrm{opt}} > C^*_{S,i,\mathrm{opt}} > C^*_{S,\mathrm{eq}} > 0$ $(i = 1, 2, \ldots, N)$ for the case where conversion of the reactant to the product is sought. The analytical approach as provided by Equations (16)–(17) assumes special significance as a short-cut method for pre-design purposes involving K_S not too different from K_P and $C^*_{S,N}$ not too much below unity if a reasonable upper bound for the relative error, Er (say, up to 20%) is acceptable (this type of reasoning can not be extended to the numerical example of the fumarase-catalyzed reaction because the degree of conversion considered, 45%, is well above the upper boundary of 10%). This approximation becomes better as both K^*_S and K^*_P increase as apparent from Figure 1. Parameter M affects the spread of the confidence contours about the exact solution lying on the diagonal of Figure 1 (i) & (ii); the larger M, the better the short-cut method for any given pair of values for K^*_S and K^*_P. As expected, at $M = 1$ the plot is symmetrical about the main diagonal. Larger values of M skew the confidence contours downwards, thus leading to more relaxed constraints on the side of positive deviations for $\mathrm{Da}_{i,\mathrm{opt}}$. The existence of horizontal and vertical asymptotes as predicted by Equations (25)–(26) is obvious in the aforementioned plots. A high practical value can then be ascribed to this analytical approximation for large values of either of the dimensionless kinetic constants because the short-cut method becomes particularly accurate in the most critical range for selection of capital investment (i.e, where the large volumes for a given conversion are required). If one seeks only a set of lower and upper bounds for the total reactor volume required for a desired conversion (e.g., during the preliminary design steps of a series of CSTR's), then Equations (17) and (22) can be employed to advantage. Further refinement of the estimates can then be obtained by numerically solving Equation (11) using the constraints introduced before.

NOMENCLATURE

C_{enz}	total concentration of enzyme [mol · m^{-3}]
$C_{P,\mathrm{eq}}$	equilibrium concentration of product [mol · m^{-3}]
$C_{P,i}$	concentration of product in the i-th reactor [mol · m^{-3}]
$C^*_{P,i}$	$(C_{P,i}/C_{S,0})$, normalized concentration of product [—]
$C_{P,0}$	concentration of product at the inlet of the first reactor [mol · m^{-3}]
C_S	concentration of reactant [mol · m^{-3}]
$C_{S,\mathrm{eq}}$	equilibrium concentration of reactant [mol · m^{-3}]
$C_{S,i}$	concentration of reactant in the i-th reactor [mol · m^{-3}]
$C^*_{S,i}$	$(C_{S,i}/C_{S,0})$, normalized concentration of reactant [—]
$C^*_{S,i,\mathrm{opt}}$	normalized concentration of reactant leading to a minimum of $\mathrm{Da}_{\mathrm{tot}}$ [—]
$C^*_{S,i,\mathrm{opt,ap}}$	normalized concentration of reactant leading to a minimum of $\mathrm{Da}_{\mathrm{tot}}$ under the assumption that $K_S = K_P$ [—]
$C_{S,N}$	concentration of reactant at the outlet of the last reactor in the series of CSTR's [mol · m^{-3}]

$C^*_{S,N}$	$(C_{S,N}/C_{S,0})$, normalized concentration of reactant at the outlet of the last reactor [—]
$C_{S,0}$	concentration of reactant at the inlet of the first reactor [mol · m^{-3}]
Da_i	$(v_{\max,f} \cdot V_i)/(Q \cdot C_{S,0})$, Damköhler number for the i-th reactor
$\mathrm{Da}_{i,\mathrm{opt}}$	Damköhler number for the i-th reactor leading to a minimum of $\mathrm{Da}_{\mathrm{tot}}$ under the assumption that $K_S = K_P$ [—]
$\mathrm{Da}_{i,\mathrm{opt,est}}$	Damköhler number for the i-th reactor leading to a minimum of $\mathrm{Da}_{\mathrm{tot}}$ under the assumption that $(K^*_P - K^*_S)C^*_{S,i} + K^*_S(1 + M + K^*_P) = K^*_P(1 + K^*_S) + MK^*_S$ [—]
Da_{pf}	Damköhler number using a plug flow reactor [—]
$\mathrm{Da}_{\mathrm{tot}}$	$\sum_{i=1}^{N} \mathrm{Da}_i$, overall Damköhler number [—]
$\mathrm{Da}_{\mathrm{tot,opt}}$	$\sum_{i=1}^{N} \mathrm{Da}_{i,\mathrm{opt}}$, minimum overall Damköhler number [—]
$\mathrm{Da}_{\mathrm{tot,opt,ap}}$	$N\ \mathrm{Da}_{i,\mathrm{opt,ap}}$, minimum overall Damköhler number under the assumption that $K_S = K_P$ [—]
$\mathrm{Da}_{\mathrm{tot,opt,est}}$	$N\ \mathrm{Da}_{i,\mathrm{opt,est}}$, minimum overall Damköhler number under the assumption that $(K^*_P - K^*_S)C^*_{S,i} + K^*_S(1 + M + K^*_P) = K^*_P(1 + K^*_S) + MK^*_S$ [—]
Dv_i	$(\mathrm{Da}_{i,\mathrm{opt,ap}}/\mathrm{Da}_{i,\mathrm{opt}} - 1)$, actual value of the fractional error originated from taking $K^*_S \sim K^*_P$ [—]
E	enzyme
Er	$(\mathrm{Da}_{i,\mathrm{opt,ap}}/\mathrm{Da}_{i,\mathrm{opt,est}} - 1)$, estimated value of the fractional error originated from taking $K^*_S \sim K^*_P$ [—]
ES	enzyme/substrate intermediate complex
i	dummy integer variable [—]
j	dummy integer variable [—]
K_{eq}	$(C_{P,\mathrm{eq}}/C_{S,\mathrm{eq}})$, equilibrium constant for the enzymatic reaction [—]
K_P	Michaelis-Menten constant for the product of the enzymatic reaction [mol · m^{-3}]
K^*_P	$(K_P/C_{S,0})$, Michaelis-Menten constant for the product [—]
K_S	Michaelis-Menten constant for the reactant of the enzymatic reaction [mol · m^{-3}]
K^*_S	$(K_S/C_{S,0})$, Michaelis-Menten constant for the reactant [—]
M	$(C_{P,0}/C_{S,0})$, ratio of initial concentrations of product and reactant [—]
N	number of reactors in the series [—]
P	product of the biochemical reaction
Q	volumetric flow rate through the reactor network [m^3 · s^{-1}]
S	reactant of biochemical reaction
t	time elapsed since start-up of the reaction [s]
v	overall kinetic rate of enzymatic reaction [mol · m^{-3} · s^{-1}]
$v_{\max,f}$	maximum rate of enzymatic reaction in the forward direction [mol · m^{-3} · s^{-1}]
$v_{\max,r}$	maximum rate of enzymatic reaction in the reverse direction [mol · m^{-3} · s^{-1}]
V	volume of reacting fluid [m^3]
V_i	liquid volume of the i-th reactor [m^3]
$V_{i,\mathrm{opt}}$	liquid volume of the i-th reactor leading to a minimum of $\mathrm{Da}_{\mathrm{tot}}$ [m^3]
$V_{i,\mathrm{opt,ap}}$	liquid volume of the i-th reactor leading to a minimum of $\mathrm{Da}_{\mathrm{tot}}$ under the assumption that $K_S = K_P$ [—]

$V_{i,\mathrm{opt,est}}$ liquid volume of the i-th reactor leading to a minimum of Da_{tot} under the assumption that $(K_P^* - K_S^*)C_{S,i}^* + K_S^*(1 + M + K_P^*) = K_P^*(1 + K_S^*) + MK_S^*$ [—]

Greek letters

ξ dummy variable of integration
ζ dummy variable of integration

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