Revisiting the Maximum Conversion of Substrate in an Enzymatic CSTR with Micromixing Considerations

F. XAVIER MALCATA

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

The balance equations pertaining to an enzyme undergoing first order thermal deactivation and a substrate undergoing enzyme-catalyzed transformation following Michaelis-Menten kinetics in a CSTR are solved for the two limits of micromixing. The maximum conversions of substrate are obtained under the assumption that the space time of the reactor is infinite. The range of the maximum conversion of substrate is wider at intermediate values for the dimensionless Michaelis-Menten constant and becomes narrower as the ratio of the time scales of the enzyme-catalyzed reaction and the enzyme deactivation increases.

On a résolu pour les deux limites du micromélange les équations d'équilibre d'une enzyme subissant une désactivation thermique de premier ordre et d'un substrat subissant une transformation catalysée par l'enzyme suivant la cinétique de Michaelis-Menten dans un réacteur agité continu (CSTR). Les conditions maximales de conversion du substrat sont obtenues en partant de l'hypothèse que l'espace-temps du réacteur est infini. La gamme de conversion maximale du substrat est plus large aux valeurs intermédiaires pour la constante adimensionnelle de Michaelis-Menten et se rétrécit lorsque les échelles de temps de la réaction catalysée par l'enzyme et la désactivation de l'enzyme augmentent.

Keywords: maximum segregation, maximum mixedness, enzyme reactor, thermal deactivation, maximum conversion.

Traditional reasoning in chemical engineering is well established that a plug flow reactor (PFR) yields higher conversions than a continuous flow stirred tank reactor (CSTR) of the same working volume in the case of chemical reactions for which the rate increases with the concentration of reactant(s) (a situation that encompasses enzyme-catalyzed reactions). However, a CSTR may be advantageous arising from the absence of temperature of concentration gradients (which may be critical for enzymes which are in general thermolabile and often inhibited by the reaction products). Furthermore, if the enzyme in question is available in bulk form for a low price, and if the reaction system will be subjected to thermal processing downstream, then immobilization is not warranted in economic terms and, thus, the use of a CSTR may become fully justified. One good example is the production of high quantities of low-lactose skim milk for lactose-intolerant people, a process in which partially pure, food grade microbial lactase is added in soluble form to milk which is incubated for some time and eventually pasteurized prior to packaging.

In dealing with industrial enzymes, the question of the stability of the enzyme is often as important as the question of activity: For this reason, in assessing whether or not a CSTR will be technically feasible for use for enzymatic reactions, one good reference value is the maximum conversion attainable with a CSTR at the desired temperature. This problem was first tackled by Malcata (1990b) for enzyme-catalyzed systems in which the conversion of substrate followed Michaelis-Menten kinetics and the deactivation of enzyme followed first order kinetics; in his approach, the condition of complete micromixing was assumed to hold. Since enzymatic systems are almost exclusively liquid systems, and a great many biochemicals increase the viscosity of water upon solubilization, the state of micromixing of the reaction system is more likely to approach the condition of maximum segregation rather than maximum mixedness. Micromixing considerations in enzymatic systems have been reported by Malcata (1987) in the form of computer software, and Malcata (1990a) in the computation of the minimum size for a cascade of CSTR's designed to achieve a desired conversion. This paper attempts to bring together the concepts of micromixing and maximum conversion of substrate using Michaelis-Menten kinetics and first-order enzyme deactivation and proves that a range of values (rather than a single value) exists for such maximum.

Theoretical analysis

The relationship between the concentration of substrate at the outlet of a CSTR and the space time τ_C under the assumption of maximum segregation, $C_{S,C,ms}$, is given by (Levenspiel, 1972)

$$C_{S,C,ms} = \int_0^\infty E_C\{t\}\ C_{S,P}\{t\}\ dt \quad \ldots\ldots\ldots\ldots\ (1)$$

where $C_{S,P}\{t\}$ is the concentration of substrate at the outlet of a plug flow reactor with space time equal to t, and $E_C\{t\}$ is the residence time distribution of the CSTR which is given by

$$E_C\{t\} = \frac{1}{\tau_C} \exp\left\{-\frac{1}{\tau_C}\right\} \quad \ldots\ldots\ldots\ldots\ (2)$$

The mass balances on active enzyme and substrate in a plug flow reactor are

$$-\frac{dC_{E,P}}{dt} = k_d\ C_{E,P}\ ;\ t = 0\ ,\ C_{E,P} = C_{E,O} \quad \ldots\ldots\ (3)$$

and

$$-\frac{dC_{S,P}}{dt} = \frac{k_r\ C_{E,P}\ C_{S,P}}{K_m + C_{S,P}}\ ;\ t = 0\ ,\ C_{S,P} = C_{S,O}\ . \quad (4)$$

respectively, where $C_{E,P}$ is the concentration of active enzyme, k_d the first order deactivation constant, k_r the first order rate constant associated with conversion of the enzyme/substrate complex into product, and K_m the Michaelis-Menten parameter, and where subscript *0* denotes inlet conditions. (In the derivation of Equations (3) and (4), the simple rate expressions based on the Michaelis-Menten mechanism for the enzyme-catalyzed transformation of substrate, and on the first order thermal deactivation of the enzyme itself were assumed to be satisfied.) Equation (3) can be integrated to yield

$$C_{E,P} = C_{E,O} \exp\{-k_d t\} \quad \ldots\ldots (5)$$

Combination of Equations (4) and (5) followed by integration allows one to obtain

$$K_m \ln\left\{\frac{C_{S,O}}{C_{S,P}}\right\} + C_{S,O} - C_{S,P} - \frac{k_r C_{E,O}}{k_d}(1 - \exp\{-k_d t\}) = 0 \quad \ldots\ldots (6)$$

After algebraic rearrangement, Equation (6) becomes

$$t = -\frac{1}{k_d}\ln\left\{1 - \frac{k_d K_m}{k_r C_{E,O}}\ln\left\{\frac{C_{S,O}}{C_{S,P}}\right\} - \frac{k_d}{k_r C_{E,O}}(C_{S,O} - C_{S,P})\right\} \quad \ldots\ldots (7)$$

Application of differentials to both sides of Equation (7) gives

$$dt = -\frac{\frac{k_d}{k_r C_{E,O}} + \frac{k_d K_m}{k_r C_{E,O}}\frac{1}{C_{S,P}}}{k_d\left(1 - \frac{k_d K_m}{k_r C_{E,O}}\ln\left\{\frac{C_{S,O}}{C_{S,O}}\right\} - \frac{k_d}{k_r C_{E,O}}(C_{S,O} - C_{S,P})\right)} dC_{S,P} \quad \ldots\ldots (8)$$

Use of Equations (2), (7), and (8) in Equation (1) finally yields

$$C_{S,C,ms} = \frac{1}{k_d \tau_C} \times \quad \ldots\ldots (9)$$

$$\times \int_{C_{S,P,min}}^{C_{S,O}} \frac{\left(\frac{k_d K_m}{k_r C_{E,O}}\frac{1}{C_{S,P}} + \frac{k_d}{k_r C_{E,O}}\right) C_{S,P}}{\left(1 - \frac{k_d K_m}{k_r C_{E,O}}\ln\left\{\frac{C_{S,O}}{C_{S,P}}\right\} - \frac{k_d}{k_r C_{E,O}}(C_{S,O} - C_{S,P})\right)^{1 - \frac{1}{k_d \tau_C}}} dC_{S,P}$$

where the minimum concentration ever attainable in a plug flow reactor, $C_{S,P,min}$, is calculated through

$$1 + \frac{k_d C_{S,O}}{k_r C_{E,O}}\frac{K_m}{C_{S,O}}\ln\left\{\frac{C_{S,P,min}}{C_{S,O}}\right\} - \frac{k_d C_{S,O}}{k_r C_{E,O}}\left(1 - \frac{C_{S,P,min}}{C_{S,O}}\right) = 0 \quad \ldots\ldots (10)$$

Equation (10) is obtained directly from Equation (7) after setting $t = \infty$.

Integration by parts allows Equation (9) be transformed into

$$C_{S,C,ms} = \left[\left(1 - \frac{k_d K_m}{k_r C_{E,O}}\ln\left\{\frac{C_{S,O}}{C_{S,P}}\right\} - \frac{k_d}{k_r C_{E,O}}(C_{S,O} - C_{S,P})\right)^{\frac{1}{k_d \tau_C}} C_{S,P}\right]_{C_{S,P,min}}^{C_{S,O}} - \int_{C_{S,P,min}}^{C_{S,O}}\left(1 - \frac{k_d K_m}{k_r C_{E,O}}\ln\left\{\frac{C_{S,O}}{C_{S,P}}\right\} - \frac{k_d}{k_r C_{E,O}}(C_{S,O} - C_{S,P})\right)^{\frac{1}{k_d \tau_C}} dC_{S,P} \quad \ldots\ldots (11)$$

In view of Equation (10), Equation (11) may be simplified to

$$C_{S,C,ms} = C_{S,O} - \int_{C_{S,P,min}}^{C_{S,O}}\left(1 - \frac{k_d K_m}{k_r C_{E,O}}\ln\left\{\frac{C_{S,O}}{C_{S,P}}\right\} - \frac{k_d}{k_r C_{E,O}}(C_{S,O} - C_{S,P})\right)^{\frac{1}{k_d \tau_C}} dC_{S,P} \quad \ldots\ldots (12)$$

The minimum value for the concentration of substrate, $C_{S,C,ms,min}$, should then be attained when the space time of the CSTR is virtually infinity, viz.

$$C_{S,C,ms,min} = C_{S,O} - \lim_{\tau_C \to \infty}\int_{C_{S,P,min}}^{C_{S,O}}\left(1 - \frac{k_d K_m}{k_r C_{E,O}}\ln\left\{\frac{C_{S,O}}{C_{S,P}}\right\} - \frac{k_d}{k_r C_{E,O}}(C_{S,O} - C_{S,P})\right)^{\frac{1}{k_d \tau_C}} dC_{S,P} \quad \ldots\ldots (13)$$

which is mathematically equivalent to

$$C_{S,C,ms,min} = C_{S,O} - \int_{C_{S,P,min}}^{C_{S,O}} dC_{S,P} \quad \ldots\ldots (14)$$

or

$$C_{S,C,ms,min} = C_{S,P,min} \quad \ldots\ldots (15)$$

a remarkably simple result.

The concentrations of active enzyme and substrate at the outlet of a CSTR with space time τ_C under the assumption of maximum mixedness, $C_{S,C,mm}$, are given by (Zwietering, 1959).

$$C_{E,O} - C_{E,C,mm} = k_d \tau_C C_{E,C,mm} \quad \ldots\ldots\ldots\ldots \quad (16)$$

and

$$C_{S,o} - C_{S,c,mm} = \frac{k_r \tau_C C_{E,C,mm} C_{S,C,mm}}{K_m + C_{S,C,mm}} \quad \ldots\ldots\ldots \quad (17)$$

respectively. Combining Equations (16) and (17) yields

$$\tau_C = \frac{(C_{S,O} - C_{S,C,mm})(K_m + C_{S,C,mm}) C_{S,C,mm}}{k_r C_{E,O} C^2_{S,C,mm} - k_d (C_{S,O} - C_{S,C,mm}) (K_m + C_{S,C,mm}) C_{S,C,mm}} \quad (18)$$

The minimum value for the concentration of substrate, $C_{S,C,mm,min}$, should then be attained when the space time of the CSTR is virtually infinity; this situation corresponds to

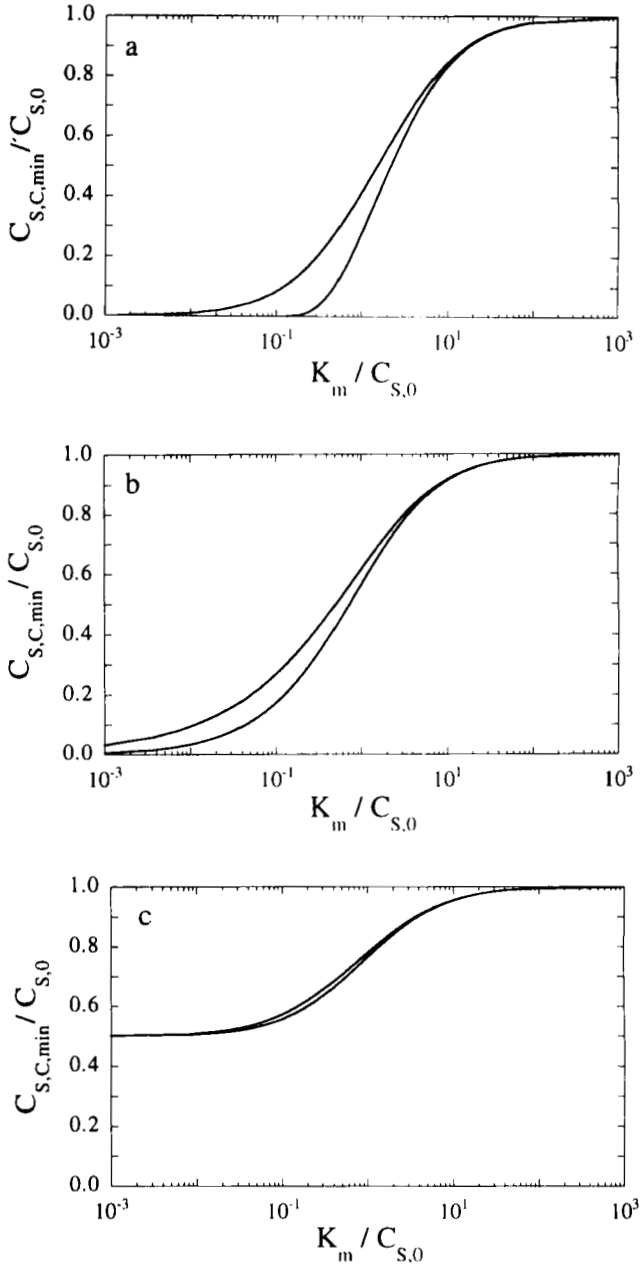
$$k_r C_{E,O} - \frac{k_d (C_{S,O} - C_{S,C,mm,min})(K_m + C_{S,C,mm,min})}{C_{S,C,mm,min}} = 0 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (19)$$

which can be manipulated to give

$$\frac{C_{S,C,mm,min}}{C_{S,C}} = \quad \ldots\ldots\ldots\ldots \quad (20)$$

$$\frac{\frac{k_d C_{S,O}}{k_r C_{E,O}} - \frac{k_d C_{S,O}}{k_r C_{E,O}} \frac{K_m}{C_{S,O}} - 1 + \sqrt{\left(\frac{k_d C_{S,O}}{k_r C_{E,O}} - \frac{k_d C_{S,O}}{k_r C_{E,O}} \frac{K_m}{C_{S,O}} - 1\right)^2 + 4 \left(\frac{k_d C_{S,O}}{k_r C_{E,O}}\right)^2 \frac{K_m}{C_{S,O}}}}{2 \frac{k_d C_{S,O}}{k_r C_{E,O}}}$$

Plots of $C_{S,C,ms,min} / C_{S,O}$ as obtained from combination of Equations (10) and (15) and $C_{S,C,mm,min} / C_{S,O}$ as obtained from Equation (20) vs. $K_m / C_{S,O}$ for several values of parameter $k_r C_{E,O}$ are available as Figures 1a through 1c.



Figures 1a - 1c — Plots of the normalized minimum concentration of substrate at the outlet of a CSTR under the assumption of maximum segregation, $C_{S,C,ms,min}/C_{S,O}$ (bottom line), and its counterpart under the assumption of maximum mixedness, $C_{S,C,mm,min}/C_{S,O}$ (top line), vs. the dimensionless Michaelis-Menten parameter, $K_m/C_{S,O}$, for: (a), $k_d C_{S,O}/k_r C_{E,O} = 0.5$; (b), $k_d C_{S,O}/k_r C_{E,O} = 1$; and (c), $k_d C_{S,O}/k_r C_{E,O} = 2$.

Discussion

In a real fluid there is, in general, partial segregation; it can be considered as a mixture of microfluid and macrofluid (Dunn and Hsu, 1973). However, the characterization of the state of mixing is not a straightforward task, and so consideration of the two limiting behaviors may prove useful from a practical point of view, especially if (as shown in the above analysis) the two asymptotic situations do not depart excessively from one another.

It is interesting to note that the difference between the maximum conversions of substrate (or, equivalently, the minimum concentrations of substrates) calculated under the assumption of maximum segregation and under the assumption of maximum mixedness (see Figures 1a - 1c) becomes smaller as parameter $k_d C_{S,O}/k_r C_{E,O}$ increases; this parameter may be viewed as the ratio of the time scale associated with the enzyme-catalyzed reaction (i.e. $C_{S,O}/k_r C_{E,O}$) to the time scale associated with the enzyme deactivation reaction (i.e. $1 / k_d$). This means that the wider range for the maximum conversion is expected to occur when no deactivation takes place, a situation which was explored elsewhere (Malcata, 1990a). In addition, it is also apparent from inspection of Figures 1a-1c that when the K_m value is either extremely low or extremely high, the state of micromixing no longer affects the maximum conversion. This means that the results reported by Malcata (1990b) can be directly employed for enzymatic reactions which can be

considered to be pseudo-zero order or pseudo-first order. For reactions with intermediate reaction orders (i.e., those for which $K_m/C_{S,O} \sim 1$), the curve describing the best conversion under the assumption of maximum segregation deviates positively from that describing the best conversion under the assumption of maximum mixedness; in such situations the expression proposed elsewhere (Malcata, 1990b) underpredicts the best conversion, so reasonings based on maximum mixedness conditions are more conservative.

The dimensionless form of the results depicted in Equations (10) and (20) is particularly useful because the values of $C_{S,C,ms,min}$ and $C_{S,C,mm,min}$ end up as being formally dependent on two parameters only, viz. $k_d\ C_{S,O}/k_r\ C_{E,O}$ and $K_m/C_{S,O}$. As expected when $k_d\ C_{S,O}/k_r\ C_{E,O}$ increases, deactivation plays a more and more important role thus preventing high conversions to be attained. One remarkable observation apparent from inspection of Equation (15) is that the conversion in a CSTR under maximum segregation conditions is virtually that a PFR, even though the RTD of a CSTR is an exponential and the RTD of a PFR is an instantaneous pulse.

It may be argued that no experimental support is provided for the rationale presented in this communication; however, it should be realized that the maximum conversion of substrate in a CSTR is reached when the size of the reactor is infinite. This infinite size is an abstraction, and experimental approaches can be developed only via extrapolating data for an existing CSTR to an infinite space time; such rationale would not be more informative than simply use well established rate expressions of enzyme performance and deactivation and well established hydrodynamic expressions for a CSTR in attempts to calculate a limiting behavior.

This work represents an addition to previous work in that the effect of the degree of micromixing was now studied in limiting operating conditions (i.e. those yielding minimum outlet concentrations) in a CSTR brought about by the existence of significant enzyme deactivation, a process which was not considered previously (Malcata, 1990a). Furthermore, this work extends previous work in that such maximum conversion of substrate was now studied in the limiting hydrodynamic conditions of maximum segregation, a micromixing limit which was not considered previously (Malcata, 1990b).

The analysis reported above is useful in the predesign steps of reactors for the performance of enzyme-catalyzed reactions which occur within time frames which are of the order of magnitude of the half-life of the enzyme utilized because it allows one to quickly estimate the range of the best possible conversions carried out by a CSTR; should this conversion be too low, no further study is warranted and alternative reactor configurations should be considered.

Nomenclature

$C_{E,P}$ = concentration of active enzyme (mol/m^3)
$C_{S,C,mm}$ = concentration of substrate at the outlet of a CSTR under the assumption of maximum mixedness (mol/m^3)
$C_{S,C,ms}$ = concentration of substrate at the outlet of a CSTR under the assumption of maximum segregation (mol/m^3)
$C_{S,C,ms,min}$ = minimum concentration of substrate at the outlet of a CSTR under the assumption of maximum segregation (mol/m^3)
$C_{S,P,min}$ = minimum concentration of substrate ever attainable in a plug flow reactor (mol/m^3)
E_C = residence time distribution of the CSTR (s^{-1})
k_d = first order deactivation constant (s^{-1})
K_m = Michaelis-Menten constant (mol/m^3)
k_r = first order rate constant for enzyme/substrate complex transformation (s^{-1})
t = dummy variable denoting residence time (s)

Greek letters

τ = space time (s)
τ_C = space time of CSTR (s)

Subscripts

O = inlet conditions

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Erratum

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In the definition of s_c in Equation (1), W_2 should be replaced by W_3, i.e., the correct definition is:

$$s_c = W_1 ss_a + W_2 ss_b + W_3$$