Strategy for the simulation of batch reactors when the enzyme-catalyzed reaction is accompanied by enzyme deactivation

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Abstract The balance equations pertaining to the modelling of batch reactors performing an enzyme-catalyzed reaction in the presence of enzyme deactivation are developed. The functional form of the solution for the general situation where both the rate of the enzyme-catalyzed reaction and the rate of enzyme deactivation are dependent on the substrate concentration is obtained, as well as the condition that applies if a maximum conversion of substrate is sought. Finally, two examples of practical interest are explored to emphasize the usefulness of the analysis presented.

List of symbols

- $C_e$ mol/m$^3$: concentration of active enzyme
- $C_{e,0}$ mol/m$^3$: initial concentration of active enzyme
- $C_s$ mol/m$^3$: concentration of substrate
- $C_{s,0}$ mol/m$^3$: initial concentration of substrate
- $C_{s,\text{min}}$ mol/m$^3$: minimum value for the concentration of substrate
- $k_1$ 1/s: first order rate constant associated with conversion of enzyme/substrate complex into product
- $k_2$ 1/s: first order deactivation constant of enzyme (or free enzyme)
- $k_3$ 1/s: first order deactivation constant of enzyme in enzyme/substrate complex form
- $K_m$ mol/m$^3$: Michaelis-Menten constant
- $p$ mol/(m$^3$s): time derivative of $C_s$
- $q$ mol/m$^3$: auxiliary variable
- $t$ s: time elapsed after reactor startup

Greek symbols

- $\phi$ 1/s: univariate function expressing the dependence of the rate of enzyme deactivation on $C_s$
- $\zeta$ mol/m$^3$: dummy variable of integration
- $\zeta'$ mol/m$^3$: dummy variable of integration
- $\psi$ 1/s: univariate function expressing the dependence of the rate of substrate depletion on $C_s$
- $\psi'$ m$^3$/mol s: derivative of $\psi$ with respect to $C_s$

1 Introduction

Batch reactors used to perform enzyme-catalyzed reactions are fairly common in the biochemical (and fine chemical) industry. In order to be able to properly design such reactors a priori and to properly optimize their utilization a posteriori, efficient techniques of mathematical modelling are required that take into account various possibilities for the kinetic mechanisms pertaining to the enzyme-catalyzed reaction and to the thermal deactivation of the enzyme. A variety of uni- and multsubstrate mechanisms for the former have been comprehensively described in the literature [1], and similar observation holds for unimolecular mechanisms associated with the latter in the presence of macroheterogeneity [2] or microheterogeneity [3]. The assumption of substrate-dependent rate expressions for either the enzyme-catalyzed reaction and the deactivation of the enzyme has been successfully employed before, e.g. in the determination of the optimum temperature path in the case of batch reactors performing enzyme-catalyzed reactions [4]. The combination of some of the aforementioned mechanisms for the enzyme-catalyzed reaction with the material balances for various reactor configurations aiming at optimized reactor designs has also been reported to considerable extent [5–10]. The existence of constraints in terms of the maximum conversion of substrate ever attainable when enzyme deactivation is present was emphasized elsewhere for the case of CSTR cascades [11–13]. However, the integration of both the enzyme-catalyzed and the enzyme deactivation reactions with the material balances pertaining to the reactor has not deserved extensive attention so far.

This communication attempts to partially fill this gap by providing a general mathematical strategy for the simulation of biochemical reactors operated batchwise when both the enzyme-catalyzed reaction and the enzyme deactivation reaction take place; its applicability is tested via the discussion of two selected examples.

2 Theory

The unsteady-state mass balances to enzyme undergoing deactivation following first order kinetics with respect to the enzyme, and substrate undergoing depletion also following first order kinetics with respect to the enzyme in a well-stirred reactor operated batchwise can be written as:

$$\frac{dC_e}{dt} = -\phi(C_s) C_s,$$

(1)
with initial condition

\[ t = 0, \quad C_S = C_{S,0}, \]

and

\[ \frac{dC_S}{dt} = -\psi(C_S) C_E, \]

with initial condition

\[ t = 0, \quad C_S = C_{S,0}, \]

respectively, where \( C_S \) and \( C_E \) are the molar concentrations of active enzyme and substrate, respectively, \( t \) is time elapsed since startup of the reactor, \( \phi \) is a univariate expression relating the rate of enzyme deactivation with substrate concentration, and \( \psi \) is a univariate expression relating the rate of substrate depletion with substrate concentration.

Equation (1) may be replaced by the expression obtained by division of Eq. (1) by Eq. (3):

\[ \frac{dC_E}{dC_S} = \frac{\phi(C_S)}{\psi(C_S)} \]

and, therefore, Eq. (2) should be replaced by:

\[ C_S = C_{S,0}, \quad C_E = C_{E,0}. \]

Rewriting Eq. (3) as:

\[ \frac{dC_E}{dt} = -\frac{1}{\psi(C_S)} \frac{dC_S}{dt}, \]

and differentiating Eq. (7) with respect to \( t \), one gets:

\[ \frac{\left( \frac{dC_E}{dt} \right)}{\left( \frac{dC_S}{dt} \right)} \psi(C_S) - \psi'(C_S) \frac{dC_S}{dt} + \frac{1}{\psi} \left( \frac{d^2C_S}{dt^2} \right) = 0, \]

where:

\[ \psi' \equiv \frac{d\psi}{dC_S}. \]

Combination of Eqs. (8) and (3) yields:

\[ \left( \frac{d^2C_S}{dt^2} \right) + \phi(C_S) \frac{dC_S}{dt} - \psi'(C_S) \frac{dC_S}{dt} = 0, \]

which now requires two initial conditions, viz. Eq. (4) and

\[ t = 0, \quad \frac{dC_S}{dt} = -\psi(C_S) C_{E,0}. \]

Equation (10) is a second-order, second-degree ordinary differential equation which can replace Eq. (3). Solution of Eq. (10) may proceed through the introduction of the new dependent variable \( p \), defined as:

\[ p \equiv \frac{dC_S}{dt}, \]

which, combined with Eq. (10), leads to [14]:

\[ \left( \frac{dp}{dC_S} \right) - \psi(C_S) p = -\phi(C_S); \]

\[ C_S = C_{S,0}, \quad p = -\psi(C_S) C_{E,0}. \]

Equation (13) has the form of a general first order ordinary differential equation, which has the following solution:

\[ p = -\psi(C_S) \left( C_{E,0} + \int_{C_{E,0}}^{C_s} \frac{\phi(C_S)}{\psi(C_S)} dC_S \right). \]

Recalling Eq. (12), one finally observes that:

\[ t = -\int_{C_{E,0}}^{C_s} \frac{\phi(C_S)}{\psi(C_S)} \frac{dC_S}{C_{E,0} + \int_{C_{E,0}}^{C_s} \frac{\phi(C_S)}{\psi(C_S)} dC_S}, \]

which can be rewritten as:

\[ t = \int_{C_{S,0}}^{C_s} \frac{1}{\psi(C_S)} \frac{dC_S}{\phi(C_S)} \frac{dC_S}{C_{E,0} + \int_{C_{E,0}}^{C_s} \frac{\phi(C_S)}{\psi(C_S)} dC_S}. \]

The auxiliary variable \( q \) is defined as:

\[ q \equiv \left( -\frac{p}{\psi} \right). \]

Recall Eq. (16), which can be written in the alternative form:

\[ t = \int_{C_{S,0}}^{C_s} \frac{dC_S}{\psi(C_S)} \frac{dC_S}{\phi(C_S)} \frac{dC_S}{C_{E,0} + \int_{C_{E,0}}^{C_s} \frac{\phi(C_S)}{\psi(C_S)} dC_S}. \]

For \( t = \infty \), one will obtain the maximum ever attainable conversion of substrate, \( C_{S,\text{min}} \). Since the integration range is finite, inspection of the denominator of the integrating function in Eq. (19) indicates that, in such situation, \( C_{S,\text{min}} \) should satisfy the following condition:

\[ \psi(C_{S,\text{min}}) = 0 \lor C_{E,0} = -\int_{C_{E,0}}^{C_s} \frac{\phi(C_S)}{\psi(C_S)} dC_S = 0. \]

The first condition is trivial, since for every type of kinetics for substrate consumption by an enzyme-catalyzed reaction, the rate of reaction is obviously nil when the concentration of substrate is zero. The second condition is much more constraining. It arises from the fact that there are two competing factors in the reacting system, the deactivation of enzyme, which leads to a decrease in the number of active enzyme molecules with time, and the rate of the enzyme-catalyzed reaction, which leads to a decrease in the number of substrate molecules with time; hence, longer reaction times imply more extensive conversion of substrate per unit molecule of active enzyme but also fewer molecules of enzyme left active, which may lead to the existence of a maximum for the conversion of substrate. It should be emphasized that \( C_{S,\text{min}} > 0 \) if \( \psi \) is a weaker function on \( C_S \) than is \( \psi \) irrespective of the value of \( C_{E,0} \), which is equivalent, in physical terms, to saying that \( \phi(C_S) \psi(C_S) \) becomes unbounded when \( C_S \) tends to zero; the equality \( C_{S,\text{min}} = 0 \) can be guaranteed a priori only if the enzyme is actually reactivated by the presence of substrate at whichever concentration, i.e. if \( \phi(C_S) < \sigma \); in the remaining cases, e.g. \( \phi(C_S) \psi(C_S) \) equal to a constant or \( \lim_{C_S \to 0} \phi(C_S) \psi(C_S) \) equal to a finite value, the decision on which solution of Eq. (20) holds depends on the numerical values of the parameters in question.

The applicability of the foregoing general analysis is explored below in two examples of practical interest.
First numerical example

Assume that the rate of deactivation is independent of the concentration of substrate. In this situation, \( \phi \{ C_S \} \) reduces to a constant, and Eq. (17) becomes:

\[
t = \left( \frac{1}{\phi \{ C_{S,0} \}} \right) \int_{q \{ C_S \}}^{C_{S,0}} \frac{d\eta}{\eta},
\]

or, using Eqs. (15) and (18):

\[
t = -\left( \frac{1}{\phi \{ C_{S,0} \}} \right) \ln \left\{ 1 - \frac{\phi \{ C_{S,0} \} C_{E,0}}{C_{S,0}} \right\} \int_{q \{ C_S \}}^{C_{S,0}} \frac{d\xi}{\xi}.
\]

Assuming Michaelis-Menten kinetics for the substrate consumption and first order deactivation of enzyme, the result is:

\[
t = -\left( \frac{1}{k_1} \right) \ln \left\{ 1 - \frac{k_1 C_{S,0}}{C_{E,0}} \right\} \int_{q \{ C_S \}}^{C_{S,0}} \frac{d\xi}{\xi},
\]

where \( k_1 \) is the first order deactivation constant, \( K_m \) is the Michaelis-Menten parameter i.e. the dissociation constant of the enzyme/substrate complex, and \( k \) is the first order constant associated with transformation of the enzyme/substrate complex into product and concomitant regeneration of the free enzyme form. After simple manipulation of Eq. (23), one finally gets:

\[
k_1 t = -\ln \left\{ 1 - \frac{k_1 C_{S,0}}{k C_{E,0}} \left( \frac{K_m}{C_{S,0}} \ln \left\{ \frac{C_{S,0}}{C_{S}} \right\} + 1 - \frac{C_S}{C_{S,0}} \right) \right\}.
\]

Plots of \( C_S/C_{S,0} \) vs. \( k_1 \) are available in Figs. 1a to 1c for a number of values of parameters \( k C_{E,0}/(k_1 C_{S,0}) \) and \( k_m/C_{S,0} \) and \( K_m/C_{S,0} \).

Second numerical example

Assume now that the free enzyme deactivates via a first order irreversible process with constant \( k_1 \), and that the enzyme in the enzyme/substrate complex form deactivates via a first order irreversible process characterized by constant \( k_2 \), which, in general, will be different from \( k_1 \); assume in addition that the substrate consumption follows Michaelis-Menten kinetics as in the previous example. In this situation, Eqs. (1) and (3) take the form:

\[
\frac{dC_S}{dt} = -\frac{k_1 K_m}{C_{S,0}} C_S + k C_S,
\]

\[
\frac{dC_E}{dt} = -\frac{k C_S}{K_m + C_S}.
\]

Equation (20) now takes the form:

\[
\int_{C_{S,min}}^{C_{S,0}} \frac{k_1 (K_m + \xi)}{k_1 C_{S,0}} d\xi = C_{E,0},
\]

which is equivalent to:

\[
\frac{k m}{C_{S,0}} \ln \left\{ \frac{C_{S,0}}{C_{S,min}} \right\} - \frac{C_{S,min}}{C_{S,0}} + 1 - \frac{k C_{E,0}}{k_1 C_{S,0}} = 0.
\]

Plots of \( C_{S,min}/C_{S,0} \) are available in Fig. 2 as functions of parameters \( k C_{E,0}/(k_1 C_{S,0}) \) and \( K_m/C_{S,0} \).
which, upon integration and rearrangement, becomes:

\[ t = - \int_{\frac{K_m + \zeta}{k \zeta}}^{\frac{K_m}{k \zeta}} C_{E,0} + \frac{k_1 K_m + k_2 \zeta}{k \zeta} \, d\zeta. \]  

(29)

Algebraic manipulation of Eq. (24) yields:

\[ t = - \frac{1}{k_1} \int_{\frac{C_{E,0}}{C_{S,0}}}^{\frac{K_m}{k \zeta}} \frac{1}{k} \frac{k_1 K_m + k_2 \zeta}{k \zeta} \ln \left( \frac{C_{S,0}}{C_{S,0}} \right) \, d\zeta + \ldots + \frac{k_2}{k} \frac{k_1 - k_2}{k} \zeta \ln \left( \frac{C_{S,0}}{C_{S,0}} \right) \]  

\[ + \int_{\frac{C_{E,0}}{C_{S,0}}}^{\frac{K_m}{k \zeta}} \frac{k_1 K_m + k_2 \zeta}{k \zeta} \ln \left( \frac{C_{S,0}}{C_{S,0}} \right) \, d\zeta, \]  

(30)

which, upon integration and rearrangement, becomes:

\[ k_1 t = - \ln \left( 1 - \frac{C_{S,0}}{C_{E,0}} \right) \ln \left( \frac{C_{S,0}}{C_{S,0}} \right) + \frac{C_{E,0}}{k_1} \left( 1 - \frac{C_{S,0}}{C_{S,0}} \right) \ln \left( \frac{C_{S,0}}{C_{S,0}} \right) + \ldots + \left( 1 - \frac{k_2}{k_1} \right) \int_{\frac{C_{E,0}}{C_{S,0}}}^{\frac{K_m}{k \zeta}} \frac{C_{S,0}}{k \zeta} + \frac{C_{E,0}}{k \zeta} \ln \left( \frac{C_{S,0}}{C_{S,0}} \right) + \frac{k_2}{k_1} \ln \left( \frac{C_{S,0}}{C_{S,0}} \right) \right) \]  

(31)

Plots of \( C_{S,0} / C_{S,0} \) vs. \( k_1 t \) are available in Figs. 3a to 3f for a number of values of parameters \( k_{E,0}, (k_1, C_{S,0}), k_2 / k_1, \) and \( k_n / C_{S,0} \).

Equation (20) takes in this situation the form:

\[ \int_{C_{S,0}}^{C_{E,0}} \frac{k_1 K_m + k_2 \zeta}{k \zeta} \, d\zeta = C_{E,0}, \]  

(32)

which can be rewritten as:

\[ \frac{K_m}{C_{S,0}} \ln \left( \frac{C_{S,0}}{C_{S,0}} \right) + \frac{k_2}{k_1} \left( 1 - \frac{C_{S,0}}{C_{S,0}} \right) \frac{C_{E,0}}{k \zeta} + \frac{k_2}{k_1} \ln \left( \frac{C_{S,0}}{C_{S,0}} \right) = 0. \]  

(33)

Plots of \( C_{S,0} / C_{S,0} \) are available in Fig. 4 as functions of parameters \( k_{E,0}/(k_1, C_{S,0}) \) and \( k_2 / k_1 \).

### 5 Discussion and conclusions

It is interesting to note that in both examples studied the minimum conversion of substrate ever attainable is a bivariate function as apparent from inspection of Eqs. (26) and (33); if one moves from example 1 to example 2, parameter \( k_{E,0}/(k_1, C_{S,0}) \) should be substituted by the lumped parameter \( k_{E,0}/(k_1, C_{S,0}) \), whereas parameter \( k_n/C_{S,0} \) should be substituted by parameter \( k_2/k_1 \). Inspection of Fig. 2 indicates that the normalized value of \( \ln(C_{S,0}) \) is essentially a linear function of parameter \( k_{E,0}/(k_1, C_{S,0}) \), with a common vertical intercept at unity and a slope that decreases as the dimensionless value of \( K_m \) increases. With respect to Fig. 4, it is apparent that (a) for \( k_2/k_1 < 0 \), \( \ln(C_{S,0}) \) is always a decreasing linear function of \( k_{E,0}/(k_1, C_{S,0}) \), and that (b) such linear behavior is also observed for other values of \( k_2/k_1 \), provided that parameter \( k_{E,0}/(k_1, C_{S,0}) \) takes large values.

Careful inspection of Eq. (31) allows one to conclude that if \( k_2/k_1 = 0 \), then example 2 reduces to example 1. This fact should be expected because it implies that the rate of deactivation of enzyme is the same irrespective of the forms it takes, and it is known that the relative amounts of either free or conjugated forms of enzyme are direct functions of the concentration of the substrate. It should be noted that reactivation of the enzyme by substrate implies that the intrinsic activity of the enzyme actually increases when substrate is present, as compared with the complete absence thereof; this is not the case explored in example 2 for \( k_2/k_1 < 1 \) because in the latter situation the presence of substrate leads only to a decrease of the rate of deactivation of enzyme when substrate exists in large amounts with respect to its complete absence. The lines depicted in Figs. 3a to 3f associated with \( k_2/k_1 = 0 \) correspond to the situation where the presence of substrate at any concentration prevents inactivation of enzyme.

In Figs. 1 and 3, the lines plotted extend from unity to a certain lower asymptotic limit that can be obtained in every case from Fig. 2 or Fig. 4, respectively. Although in a few of the several cases depicted in Figs. 1 and 3 it seems that the lines extend virtually to zero substrate concentration, such is never the case because the value of \( C_{S,0} \) is actually finite and positive, as implied by the fact that \( \phi \) is always a weaker function on the enzyme than is \( \psi \) in all the cases depicted. As expected, when \( k_{E,0}/(k_1, C_{S,0}) \) increases, i.e. when either \( k/k_1 \) or \( C_{S,0}/C_{E,0} \) increases, the minimum concentration of substrate attainable decreases because the effect of \( k_1 t \) on the enhancement of the rate of the enzyme-catalyzed reaction is stronger than said effect on the enhancement of the enzyme deactivation reaction. Given any set of values for \( k_{E,0}/(k_1, C_{S,0}) \) and \( k_n/C_{S,0} \), both increases of \( k_2/k_1 \) from zero to unity, and from unity to a higher value lead to decreases in \( C_{S,0} \), thus implying that detrimental effects of the presence of substrate on the activity of the enzyme always constrain the best performance of the batch reactor.

Furthermore, the effect of \( k_2/k_1 \) is diluted when \( k_{E,0}/C_{S,0} \) and \( k_{E,0}/(k_1, C_{S,0}) \) are large.

The analysis presented in this communication is relevant for the simulation of batch reactors that perform enzyme-catalyzed reactions because it includes the effect of thermal deactivation, a factor that very often places severe constraints on the technical and commercial feasibility of such reactors. The reasoning was developed in such way as to possess the advantage of being...
Fig. 3a-f. Plots of the dimensionless substrate concentration, $C_S/C_{S,0}$, vs. the dimensionless time, $k_1 t$, for: (a) $k_{C,0}/(k_1 C_{S,0}) = 1$ and $K_m/C_{S,0} = 0.1$; (b) $k_{C,0}/(k_1 C_{S,0}) = 1$ and $K_m/C_{S,0} = 1$; (c) $k_{C,0}/(k_1 C_{S,0}) = 1$ and $K_m/C_{S,0} = 10$; (d) $k_{E,0}/(k_1 C_{S,0}) = 10$ and $K_m/C_{S,0} = 0.1$; (e) $k_{E,0}/(k_1 C_{S,0}) = 10$ and $K_m/C_{S,0} = 1$; and (f) $k_{E,0}/(k_1 C_{S,0}) = 10$ and $K_m/C_{S,0} = 10$.

Fig. 4. Log-lin plots of the dimensionless minimum substrate concentration, $C_{S,\text{min}}/C_{S,0}$, vs. the dimensionless parameter $[k_{E,0}/(k_1 C_{S,0})]/(K_m/C_{S,0})$ for $k_2/k_1 = 0$, $k_2/k_1 = 5$, $k_2/k_1 = 12$, and $k_2/k_1 = 20$.

dimensionless, and having the final form of the solution of the simultaneous material balances to substrate and enzyme, viz. Eq. (19), require evaluation of an integral rather than solution of a set of two differential equations, viz. Eqs. (1) and (3).

References