Maintaining constant enzyme activity in a continuous flow reactor

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Abstract

The feed rate of make-up enzyme into a continuous flow enzyme reactor is theoretically determined to offset exactly the loss of activity through enzyme deactivation. An expression for the feed rate is developed for arbitrary deactivation kinetics, and then applied to two realistic deactivation mechanisms for which analytical solutions are developed. When a first-order deactivation mechanism is used, it is found that the make-up enzyme must be fed into the reactor at a constant rate to maintain constant enzyme activity. When a two-step deactivation scheme applies, the corresponding feed rate is an increasing function of time. These results can be used to simplify the study of enzyme kinetics as well as to specify conditions for generating a useful product at a constant rate from an enzyme reactor for which enzyme deactivation cannot be neglected.

1. Introduction

Enzyme deactivation, which plays an important role in the use of enzyme reactors [1] — whether for kinetic studies or for the manufacture of commercially useful products — can be accounted for in two ways. The first approach is to account explicitly for the deactivation kinetics [2]. The alternative approach of introducing fresh enzyme into the system so as to compensate exactly for the loss of activity through deactivation is examined theoretically in the context of determining intrinsic enzyme kinetics using a membrane reactor [3]. Such a reactor is shown schematically in Fig. 1. The theoretical nature of the present treatment is emphasized, where Fig. 1 serves to illustrate how the approach discussed could be carried out experimentally.

2. Theoretical approach

In the apparatus shown in Fig. 1 the fresh enzyme is added at the mass flow rate \( \dot{E}(t) \) so as to ensure that the total activity within the reactor remains constant at its initial value of \( A_0 \). The specific activity within the reactor decreases with time because the amount of enzyme (i.e., protein concentration) is increased at various levels of deactivation. However, the specific activity of the fresh enzyme in the reservoir remains constant at its initial value of \( a_0 \), since it is assumed that deactivation only occurs under the conditions found in the reactor. In Fig. 1, the deactivation is assumed to be caused by temperature, but the argument that follows is more generally applicable.

The total activity in the reactor at any time, \( \dot{A}(t) \), may be conveniently considered in terms of two contributions: one from the enzyme initially present in the reactor, and the other from the make-up enzyme added up to that time. The first term can be determined from

\[
A(t) = A_0 D(t)
\]

where \( \dot{A} \) is the total activity, at time \( t \), of the enzyme initially present in the reactor,
$D(t)$ is the deactivation rate. In order to calculate the second term, it is noted that, on entering the reactor, the make-up enzyme also undergoes deactivation, resulting in a distribution of ages within the reactor. Make-up enzyme entering the reactor at a time $t^*$ contributes a total activity of $a_0 E(t^*) dt^*$ at time $t^*$, and a total activity of $D(t-t^*) a_0 E(t^*) dt^*$ at an arbitrary later time $t$. Thus the second term becomes $\int_0^t a_0 E(t^*) D(t-t^*) dt^*$. $E(t)$ is then found from

$$A_0 D(t) + \int_0^t a_0 E(t^*) D(t-t^*) dt^* = \tilde{A} = A_0$$  

(2)

Using eqn. (1), this can be rewritten as

$$A_0 \frac{dA}{dt} + \int_0^t a_0 E(t^*) A_0 (t-t^*) dt^* = A_0$$  

(3)

Since the integral is of convolution form, the Laplace transform can be applied to eqn. (3), giving

$$\tilde{A}(s) + \frac{a_0}{A_0} \tilde{E}(s) \tilde{A}(s) = \frac{A_0}{s}$$  

(4)

or

$$\tilde{E}(s) = \frac{A_0 a_0 - s \tilde{A}(s)}{a_0} s \tilde{A}(s)$$  

(5)

For a specified deactivation mechanism, which fixes $A_0(t)$, inverting eqn. (5) then gives the required rate $E(t)$. The situation is shown schematically in Fig. 2 for an arbitrary deactivation mechanism, for which $A_0(t)$ decreases as shown. Fresh enzyme is introduced at the rate $E(t)$ so as to offset this loss and maintain the total activity of the reactor at $A_0$ for all time.

3. Deactivation mechanisms

3.1. First-order deactivation

This is the most commonly used description of deactivation, e.g., Laider and Bunting [4].

$$\frac{dA}{dt} = -\lambda A$$  

where $\lambda = \frac{dA_0}{dt}$

and eqn.

$$A_0 e^{-\lambda t}$$

While $t$ transforms a more time (8)

$$1 + \frac{G(t)}{A_0}$$

which

$$E(t)$$

Thus $t$ be for order $A_0$ with this re-

3.2. Some describe (5-7),

$E_0 \frac{a_0}{A_0}$

where internal

$$\frac{dA}{dt} = \frac{dA_0}{dt} - \frac{dA^*}{dt}$$

with $t$. This is

$$A(t) = A^*(t)$$

Equal
for which
\[
\frac{dA}{dt} = -k_4 A
\]  
where \(k_4\) is the deactivation rate constant, or \(A = A_0 e^{-k_4 t}\). Then,
\[
A(t) = A_0 e^{-k_4 t}
\]
and eqn. (3) becomes
\[
A_0 e^{-k_4 t} + \int_0^t E(t')\mu_{A0} e^{-k_4(t-t')} dt' = A_0
\]
While the general method of taking the Laplace transform previously described could be used, a more direct approach is possible here. Equation (8) can be rewritten as
\[
1 + \frac{\alpha_0}{A_0} \int_0^t E(t')e^{\alpha_0 t'} dt' - e^{\alpha_0 t'} = 0
\]
which upon differentiation yields
\[
E(t) = \frac{\alpha_0}{A_0} k_4
\]
Thus fresh enzyme, of specific activity \(\alpha_0\), must be fed into the reactor at a constant rate in order to maintain a constant total activity of \(A_0\) within the reactor. Atkinson [2] mentions this result without proof.

3.2. Sequential deactivation
A sequential model has been proposed to describe the deactivation of some enzymes [5-7],
\[
E_1 \xrightarrow{k_{11}} E_2 \xrightarrow{k_{21}} E_d
\]
where each step is first-order and \(E_k\) is an intermediate state. Thus,
\[
\frac{dA}{dt} = -k_4 A
\]
\[
\frac{dA^*}{dt} = k_1 A - k_2 A^*
\]
with the initial conditions \(A = A_0, A^* = 0\) at \(t = 0\). This linear system can be easily solved to yield
\[
A(t) = A_0 e^{-k_4 t}
\]
\[
A^*(t) = A_0 \frac{k_1}{k_2 - k_1} (e^{-k_4 t} - e^{-k_2 t})
\]
Equation (3) can again be used, with
\[
A(t) = A(t) + A^*(t)
\]
The Laplace transform of \(A(t)\), from eqns. (13)-(15) is
\[
\hat{A}(s) = \frac{\alpha_0}{k_2 - k_1 (s + k_1)} \left( \frac{k_3}{s + k_2} - \frac{k_1}{s + k_2} \right)
\]
\[
= \alpha_0 \frac{s + (k_2 + k_1)}{(s + k_3)(s + k_2)}
\]
There follows from eqns. (5) and (16), after some manipulation,
\[
\hat{E}(s) = \frac{\alpha_0}{A_0} \frac{k_1 k_2}{s(s + (k_2 + k_1))}
\]
and, upon inversion,
\[
E(t) = \frac{\alpha_0}{A_0} \frac{k_1 k_2}{k_2 + k_3} \left(1 - e^{-(k_2 + k_3)t}\right)
\]
Fresh enzyme must therefore be fed at an increasing rate to maintain constant total activity.

4. Average specific activity in reactor
In all cases the specific activity of the enzyme within the reactor decreases with time, since the total activity is kept constant, and the total amount of enzyme increases due to the addition of fresh protein. The specific activity \(a(t)\) is related to the rate of addition of fresh enzyme by
\[
\frac{a(t)}{\alpha_0} = \frac{1}{1 + (\alpha_0/\alpha_0) \int_0^t E(t)dt}
\]
For first-order deactivation, this becomes
\[
a(t) = \frac{\alpha_0}{1 + k_4 t}
\]
5. Conclusions

The feed rates of make-up enzyme necessary to compensate exactly for the loss of activity through enzyme deactivation in a membrane reactor have been calculated analytically for two deactivation mechanisms. The first case treated, that of first-order deactivation, is by far the most common functional form found in the literature, and the particularly simple result obtained here is therefore of potential significance both in the context of measuring intrinsic enzyme kinetics, as in the membrane reactor discussed by Alfani et al. [3], and in the production of useful compounds. The use of this approach in an actual process may be limited by the steady accumulation of protein within the reactor, which could eventually either foul the membrane or result in incomplete mixing, all of the active protein not being instantaneously accessible. Further, the steady addition of protein could result eventually in a highly viscous solution. This would make stirring difficult, and could generate local thermal non-uniformities through increased energy dissipation. It then becomes necessary to regenerate the reactor and membrane periodically.

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References

Appendix A: Nomenclature

\( a(t) \) specific activity within the reactor at time \( t \) (IU g\(^{-1}\))
\( a_0 \) initial specific activity within the reactor (IU g\(^{-1}\))
\( A(t) \) total activity at time \( t \) of the enzyme initially present within the reactor (IU)
\( A_0(t) \) total activity within the reactor at time \( t \) (IU)
\( A_0 \) initial total activity within the reactor (IU)
\( D(t) \) deactivation rate (dimensionless)
\( E_a \) active form of enzyme E
\( E^* \) intermediate state of enzyme E
\( E_d \) deactivated form of enzyme E
\( k_0 \) deactivation rate constant in the first-order deactivation mechanism (s\(^{-1}\))
\( k_1 \) deactivation rate constant in the sequential deactivation mechanism (s\(^{-1}\))
\( k_2 \) deactivation rate constant in the sequential deactivation mechanism (s\(^{-1}\))