APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY 7, 11–18 (1982)

Choosing An Enzyme Reactor

Theoretical Background and Considerations as Illustrated in a Case Study

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Accepted February 18, 1981

Abstract

A short review of different types of enzyme reactors and factors of influence on the reactor choice is given. The background of their kinetic differences is discussed. The conversion of starch to maltose by use of an immobilized two-enzyme system has been chosen as a case study.

Index Entries: Enzyme reactor, choosing an; reactor, choosing an enzyme; economic evaluation, of enzyme reactors; enzyme kinetics, in choosing an enzyme reactor; reactor, kinetics and designs.

Introduction

The intention of this paper is to enlighten the reader about some of the factors of influence, with special reference to reaction and reactor kinetics, in the selection of the most suitable enzyme reactor (*1–4*) for a process.

The three generally important elements—"investment and operating costs," "process characteristics," and "reaction and reactor kinetics"—can be further subdivided into many parameters that affect the choice of an enzyme reactor. Enzyme loading, i.e., the catalytic density, directly influences the reactor volume and hence the size of the investment, whereas enzyme cost and stability, as well as the labor costs, affect operating expenses. The easiness with which the catalyst can be regenerated or replaced must also be considered, as well as other flexibility requirements. If the reactor is of multipurpose type, the depreciation cost may be re-

0273–2289/82/0912–0011$02.00

duced to a minimum for the process under consideration. A continuous operation usually requires a greater investment than a simple batch procedure. On the other hand, costs for process control and maintenance are generally lower. Operational disturbances of any type may be more serious in one type of reactor compared to the other, thus increasing the operating costs.

The nature of the process may quite often require special attention in the choice of reactor. In cases where a third phase, namely the gas phase, is involved in the already heterogenous catalytic system, special notice must be taken of mass transfer problems. Often a well-mixed system is required. This is also the case when the substrate contains solid particles or when the reaction requires accurate pH or temperature control. In cases where the substrate or product are unstable, intermittent feeding possibilities or a short residence time may be required. The latter can only be achieved in a system with a high catalyst density. If for one reason or the other a uniform product is desirable, a reactor with a plug-flow characteristic is required in a continuous process.

Contrary to those factors dicussed thus far, which all require knowledge of the process under consideration, the influence of the reaction kinetics and reactor performance on the choice of enzyme reactor type, can be described in general terms. Theoretical predictions of the expected productivity and yield, and their time dependence, can be derived from knowledge about basic kinetic data of the reaction and the reactor.

A variety of reactor constructions have been made and even more suggested. In Fig. 1 the three most commonly used, and from the kinetic point of view, basic, reactor types can be seen, the "batch"-wise-operated well-stirred reactor, the "continuous-stirred tank reactor," also called "backmix reactor," and the "packed-bed," "fixed-bed," or "plug-flow reactor." In a reactor of the last type, every substrate molecule has by definition one and the same residence time, whereas a certain time distribution exists in the "backmix reactor type." A mean residence time in the latter case is defined by the ratio of reactor volume to flow rate. All the other enzyme reactors shown in Fig. 1 have flow patterns that lie somewhere between the ideal "well-mixed" and "plug-flow" characteristics. The "expended bed" reactor is often used when the pressure drop in the "plug-flow" reactor becomes too high. If the upward flow is increased, the reactor becomes "fluidized," and the degree of backmixing increases at the same time. In this reactor a good heat and mass transfer can be obtained, even if the energy for keeping the bed fluidized sometimes can be rather large. A "recycle reactor" is often used when an insoluble substrate is to be processed, such as in the hydrolysis of starch. The unprocessed substrate can easily be separated from the product stream and recycled to the substrate flow again. A multistage reactor is a construction where every little segment behaves like an ideal "backmix reactor," but where the total flow pattern can be close to a "plug-flow" type, all depending on the number of backmixing segments. This reactor type is advantageous in cases where the reaction kinetics favor a "plug-flow" system, but where the practical circumstances speak for a well-mixed reactor. Many types of tubular reactors have been suggested in which the enzyme will be immobilized on the inner walls of the tubes. Soluble enzymes, or enzymes bound to a soluble polymer, can be used in a

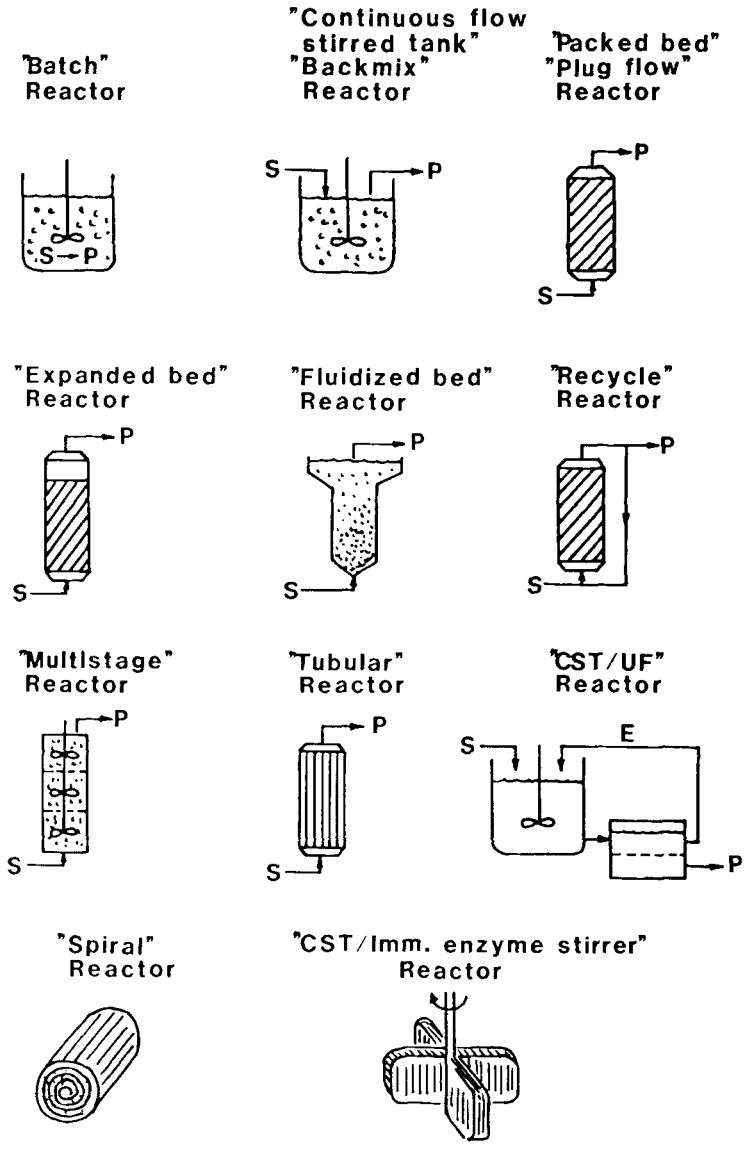

Fig. 1. Examples of reactor systems for immobilized enzymes/cells.

"continuous-stirred tank reactor" to which an ultrafiltration unit has been coupled, thus immobilizing the enzyme in the reactor system. Finally, the "spiral reactor" and the "immobilized enzyme stirrer" are examples of reactor systems where one of the primary aims has been to reduce the limitations arising from mass transfer restrictions as much as possible, without running the risk of cracking the immobilized enzyme particles.

When considering the effect of the reaction kinetics on reactor efficiency, it is most instructive to compare the three basic, and kinetically "extreme," reactor types mentioned earlier. In the most simple case, the reaction obeys the irreversible Michaelis-Menten rate equation where the reaction velocity is a function of the rate constant (k_3) for decomposition of the enzyme-substrate complex (ES), the amount

of catalyst (E), the substrate concentration (S), and the Michaelis-Menten concentration constant (K_m). In the batch reactor, where the initial substrate concentration (S_o) is converted to product, with a certain degree of conversion (X), during the total process time (t), the reaction rate at a certain time is $-ds/dt$. The same rate expression is valid for a "plug-flow" reactor. fed with a certain flow rate (Q), where different reaction velocities exists over the whole length of the reactor. In a "backmix" system the reaction velocity is the same in every part of the reactor. A simple balance over the whole reactor thus gives the reaction rate to S_oX/t. Since the kinetics of the "batch" and "plug-flow" reactors are identical in form, it is convenient to compare the "plug-flow" and the "backmix" reactors in the discussions to follow.

The following performance equations for the two reactor types can be derived from the respective rate expressions.

$$\text{Plug-flow reactor: } k_3E/Q = S_oX - K_m \ln (1 - X)$$
$$\text{Backmix reactor: } k_3E/Q = S_oX + K_m [X/(1 - X)]$$

If $S_o >> K_m$ and X is not close to 1, the reaction becomes of zero order with respect to substrate concentration, and the two performance equations become identical. If $S_o >> K_m$ or X is close to 1, however, the reaction rate is first order, and the performances of the two reactors are different. It is worth noticing that the degree of conversion in most practical processes is greater than 90%, and often close to 100%. A comparison of the efficiencies of the two reactors for conversions of 90 and 99%, respectively, indicates that 4 and 22 times, respectively, more enzyme are required in the "backmix" reactor compared to the "plug-flow" reactor. The more the ratio S_o/K_m decreases and X increases, the greater becomes the relative efficiency of the "plug-flow" reactor compared to the "backmix" reactor.

Many enzymes are subject to inhibition by the substrate or the product of the process. In a similar manner, performance equations can be derived for these systems. When the catalyst is inhibited by the substrate, this affects the productivity much more seriously in a "plug-flow" reactor compared to a reactor of "backmix" type. This seems quite understandable since the "backmix" reactor usually operates at a low substrate concentration. However, it has been shown that substrate-inhibited reactions, carried out in a "backmix" reactor, may display more than one steady-state, and some degrees of conversion may be impossible to reach. When the reaction suffers from product inhibition, the opposite situation is true. In this case the "plug-flow" reactor becomes even more favorable compared to the "backmix" reactor, since all the catalyst is exposed to the final product concentration in the latter case, whereas this is the case only in the outlet part of the "plug-flow" reactor.

The observed kinetics of immobilized enzymes is very often influenced by mass transfer effects, in the form of external and internal diffusional resistance. Many mathematical treatments have been made, in order to describe the behavior of those complications in different reactor systems, but this matter will not be treated here. Generally it can be stated that a diffusional resistance of any type is shown in an increase in the apparent K_m. Provided that the diffusional limitations are of the same size in both a "plug-flow" and a "backmix" reactor, the effect of the restric-

tions will be more serious in the latter reactor type. The reverse is true if the system is inhibited by the substrate. However, it should be noted that in practical cases the external diffusional resistance is often more pronounced in a "plug-flow" reactor than in a well-mixed system.

Another important factor that has a direct influence on process kinetics is enzyme stability. The enzyme decay during operation can be represented by a first-order process, where the loss of enzyme activity is proportional to the amount of enzyme present at a certain time. The proportional factor is called the decay constant (k_D). When zero-order kinetics are valid, the "plug-flow" and "backmix" reactors are identically affected by a loss in enzyme activity during operation. However, the change in the degree of conversion in the former reactor type is more sensitive to enzyme inactivation than in the latter reactor type when the kinetics are first-order. It is, however, again worth recalling that a greater amount of enzyme is required in the "backmix" reactor compared to the reactor of "plug-mix" type for one and the same degree of conversion. Another fact that cannot be ignored is that many enzymes are stabilized by their substrates, and hence in those cases a higher operational stability can be expected in a plug-flow system compared to a well-mixed one.

Case Study

To illustrate the preceding kinetic discussion with a case study, I want to take the conversion of starch to maltose by use of an immobilized two-enzyme system of β-amylase and pullulanase, as an example (*5*). In this system both enzymes are markedly stabilized in the presence of starch. The enzyme decay approximately follows a first-order process. Furthermore the reaction product is a competitive inhibitor for the enzyme system. The following performance equation was constructed where all the kinetic parameters including the decay constant, previously had been determined in separate, batch-wise experiments.

$$k_3 E_o \left[\frac{1 - e^{-k_D t}}{k_D} \right] = K_m \left[\frac{1 + 342\, S_o\, (1 - X_o)}{324\, K_{ip}} \right] \ln \frac{1 - X_o}{1 - X} + S_o(X - X_o)\left(1 - \frac{342}{324} \frac{K_m}{K_{ip}}\right)$$

A comparison between the observed and predicted behavior in a batch conversion (Fig. 2), indicated that there still existed some factor that had not been considered and that lowered the practical conversion rate, especially at higher degrees of conversion. This was probably an effect of an increased K_m for the enzyme system as the reaction preceeded owing to the lower affinity for low molecular weight dextrins. In order to get a performance equation that worked phenomenologically, it was a practical effort to replace k_3 with $k_3 f(X)$, where $f(X)$ was computed to compensate for the observed difference. Performance equations were thereafter derived for a "plug-flow" and a "backmix" reactor, respectively. The predictions were

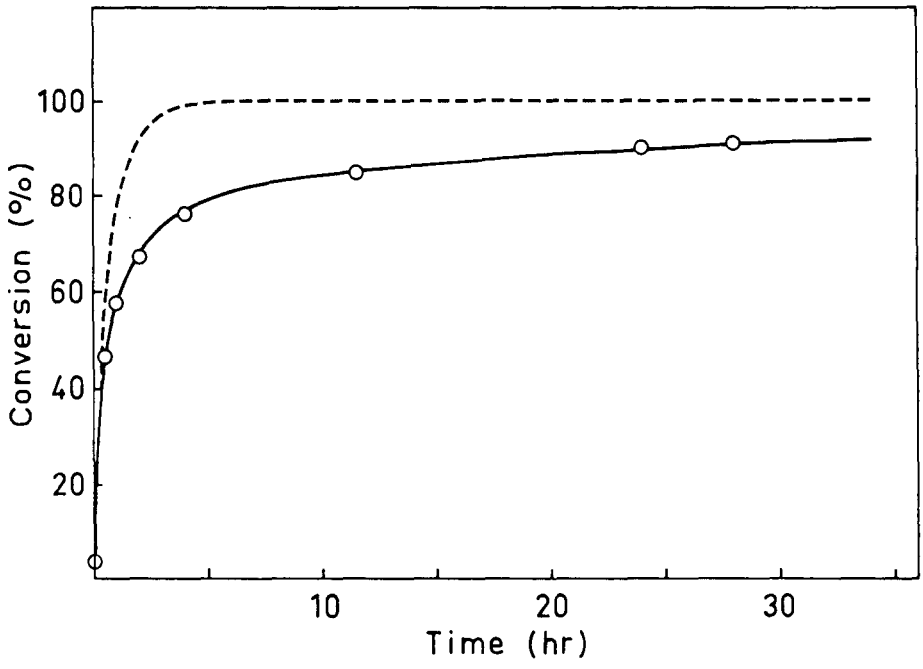

Fig. 2. Comparison of observed (solid line) and predicted (dashed line) batch conversion.

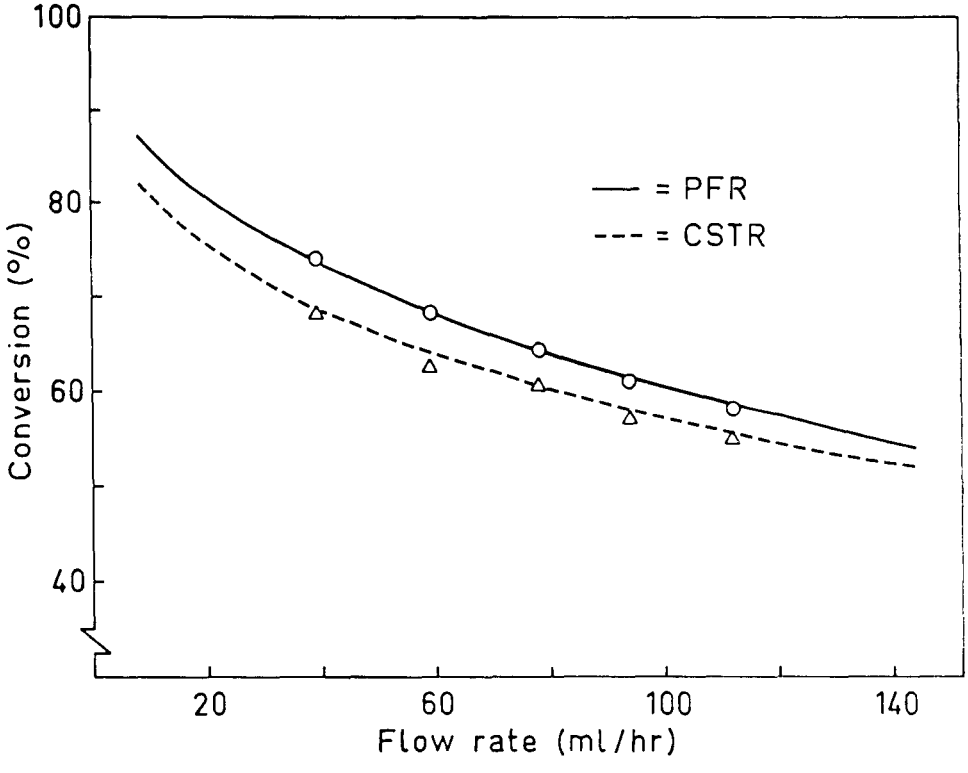

Fig. 3. Comparison of observed (o, Δ) and predicted (lines) conversions in a PFR and CSTR.

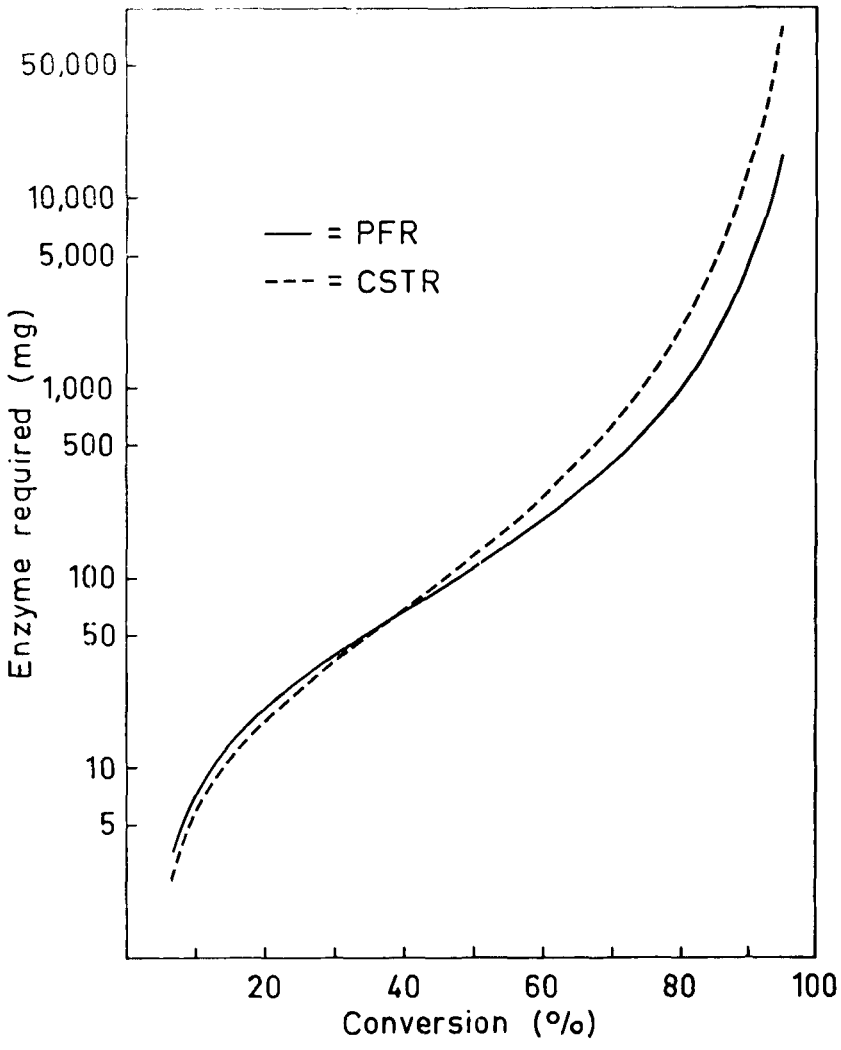

Fig. 4. Comparison of the relative efficiencies of a PFR and CSTR.

confirmed by practical experiments where a good fit was obtained (Fig. 3). It can be seen (Fig. 4) that at all degrees of conversion of interest, the theoretical predictions and practical experiments, seemed to favor the use of a "plug-flow" reactor in this case.

Even if many other factors must be considered before a definitive choice of enzyme reactor is made, a careful analysis of the reaction kinetics of the process and of phenomenologically operative performance equations can be of great practical value.

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