Enzyme Reactor Engineering*

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Summary

Design procedures for enzyme reactors do not differ fundamentally from those of conventional catalytic reactors. Initially, the decision must be made whether continuous operation has economic advantages over batchwise processing. Generally, a continuous flow reactor can be expected to yield a more uniform product and lend itself to scale-up. Control of the reaction environment in a continuous system is possible by holding the reactor at its steady-state conditions.

The type of continuous reactor chosen will depend primarily on the sensitivity of the reaction to substrate and product concentrations. Knowing the exact form of the reaction rate expression enables the chemical engineer to perform mass balance calculations which will provide quantitative comparison of the performance of different reactor designs. Special attention is necessary when evaluating laboratory kinetic data and making design calculations if the enzyme is immobilized on a solid support. Such two-phase catalytic systems are often influenced by mass diffusion rates. Chemical engineering principles can be applied to understand and quantitatively estimate the influence of process variables on diffusion rates.

Membrane enzyme reactors provide the possibility of retaining a mobile and soluble enzyme within a flow reactor. This design calls for a tank-type reactor equipped with a semipermeable membrane on the reactor outlet. The membrane permeability characteristics are selected to hold the larger enzyme molecules within the reactor while at the same time allowing the smaller product molecules to leave the reactor.

Evaluation studies and reaction rate data with immobilized enzymes under controlled mass transfer conditions can be conveniently performed using a differential-bed recirculation-loop reactor. In this laboratory reactor a large volume of fluid is continuously pumped around a tubular loop and through a small bed of supported enzyme catalyst. The pumping rate will determine the mass diffusion rates the catalyst surface. The sensitivity of the apparent reaction rate to changing flow conditions can be easily and reproducibly investigated.

Enzymes are biological catalysts which are capable of selectively and efficiently promoting chemical conversions. Enough is known about them that they can be isolated and employed to produce useful products on a commercial scale. Industrial use of enzymes is, however, not progressing as fast as some of the early workers in the field had forecasted. Scientists sometimes forget that a new commercial venture must be economically attractive. The high costs of the enzyme catalyst, associated with its preparation, the cost of carrier material and its lack of stability under reaction conditions, has slowed the development of large-scale enzyme processes. At present only two processes are producing products through immobilized enzyme catalyzed reactions.

Various aspects of enzyme reaction engineering have been treated in a number of publications[1, 2, 3, 4, 5]. Enzyme reactor design and analysis have been covered in in various reviews[1, 2, 4, 5, 6, 7]. Modelling and simulation of enzyme reaction phenomena is discussed in a recent paper[8]. An appraisal of the commercial future for enzymes has been given in an extensive report[9].

The purpose of the present paper is to discuss the design of enzyme reactors. The points that will be covered include: choosing between batch or continuous operation, selecting the reactor, consideration of the mass diffusion influence and discussion of two novel enzyme reactor designs.

Design procedures for an enzyme catalyzed process do not differ fundamentally from those used to design a conventional chemical process. Market analyses are compared with preliminary economic evaluations of the overall process. If prospects of profitably selling the product look attractive, detailed calculations are made for each piece of equipment with particular attention being given to the reactor and separations equipment. The present discussion will be limited to reactor design considerations.

Initially, a rather fundamental decision must be made as to whether the reactor will operate batchwise or continuously. All too often the decision is based solely on the desired production rate and on past experience with the reaction in the laboratory. From production rate considerations, the increased investment for a continuous process may not appear to be economically attractive. This conclusion often involves using information on product yield and catalyst stability which are often obtained from pilot or bench-scale batch equipment. This approach can lead to wrong decisions because the conditions in the batch laboratory reactor may differ considerably from those in any continuous full-scale equipment. Figure 1 indicates some of the important differences between a batch reactor and a continuous tank-type reactor. Of importance here is that whereas the batch system conditions are continuously changing from startup to shutdown, the continuous reactor can operate with steady conditions after the initial startup transient period. The continuous reactor shown here would probably operate very near to the final conditions, just before shutdown, of the batch reactor. Thus the conversion rate, yield and enzyme stability may be considerably improved in the continuous reactor. Laboratory data are very essential in designing a chemical reactor. Misinterpreted experiments on the other hand can cause one to make the wrong decision in choosing between a batch or continuous reactor. To avoid this situation, the engineer who is responsible for the reactor design should be involved in the early laboratory evaluations. In making the "batch or continuous?" decision,

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economic comparison needs to be made between the higher capital investment for the continuous reactor and the higher labour costs in operating the batch reactor.

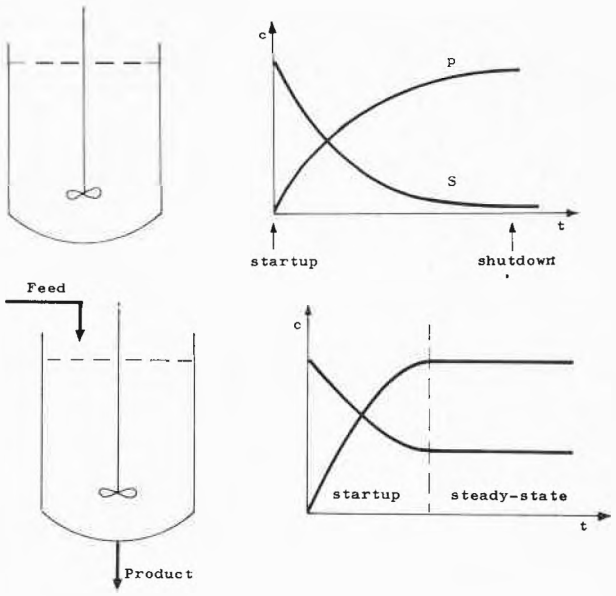


Fig. 1. Batch or continuous?

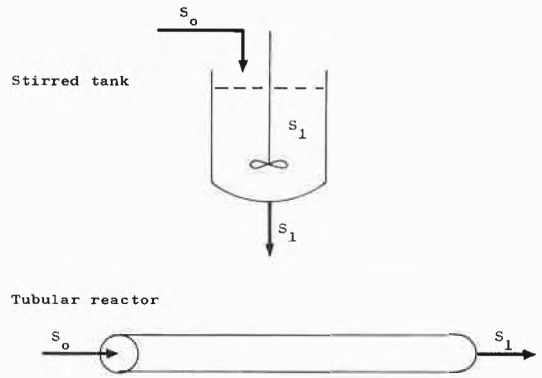


Fig. 2. Basic reactor types

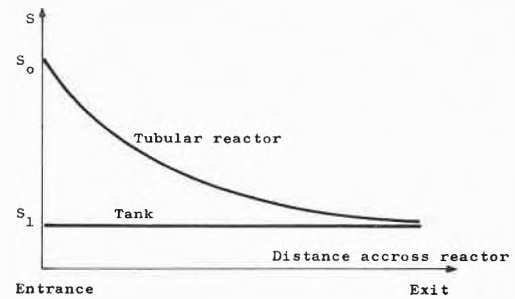


Fig. 3. Comparison of concentration gradients

Continuous reactors can be grouped into two basic idealized types: the well-mixed tank and the plug-flow tubular reactor. These are shown in Figure 2. The reaction conditions within these two systems are fundamentally different and represent two extremes: complete mixing within the tank reactor and a complete absence of mixing within the ideal tubular reactor. Consequently, conditions within the tank are the same as the outlet stream, while the conditions within the tubular reactor vary with length from inlet to outlet. This character is portrayed in Figure 3 where the substrate (reactant) concentration is plotted *versus* the distance across the reactor. Normally, the tank reactor conditions will be the same as the outlet stream from the tubular reactor. Thus, the tank reactor will operate under uniform conditions of low substrate and high product concentration. The tubular reactor conditions are never uniform, and the average conditions will be high substrate and low product concentrations. Knowledge of these reactor differences, combined with the reaction rate as a function of substrate and product concentrations, allows the design engineer to qualitatively predict which reactor type will operate most efficiently.

It is never possible to predict the reaction rate model and to calculate the kinetic constants from theory. This information must be provided by carefully planned laboratory experiments. Often the kinetics of the enzyme catalyzed reaction will follow either the Michaelis-Menten model or the product inhibition model.

Michaelis-Menten:

$$E + S \rightleftarrows ES \rightleftarrows E + P,$$

which with appropriate assumptions leads to the rate expression

$$r_P = -r_S = \frac{v_m \cdot S}{K_m + S}.$$

Product Inhibition:

$$E + P \rightleftarrows EP,$$
$$E + S \rightleftarrows ES \rightleftarrows E + P,$$

which gives the rate expression

$$r_P = -r_S = \frac{v_m \cdot S}{K_m + S + \frac{K_m}{K_I} \cdot P}.$$

For the design engineer a knowledge of the reaction model upon which the rate expression is based is not necessary. An empirical reaction rate expression which is valid under all anticipated reactor conditions is suitable. Normally however, one has more confidence in a rate expression which is based on fundamental theory.

The above rate models show that the rate of product formation, r_p, is a certain function of the product and substrate concentrations, P and S. In Figure 4 the reaction rates for Michaelis-Menten (M-M) and product inhibition kinetics are plotted *versus* substrate concentration for constant values of the rate constants v_m, K_m and K_I, and the product concentration P. Qualitatively it is seen that the Michaelis-Menten rates follow a linear expression for low S and exhibit a maximum of v_m for

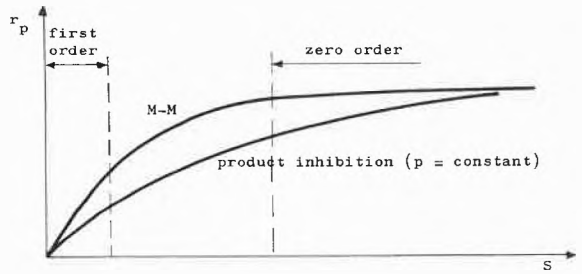


Fig. 4. Sensitivity of enzyme reaction rates to substrate concentration

high S. A product inhibition reaction system would exhibit rates which decrease with increasing product concentration.

Many of the known enzyme reactions are described by the Michaelis-Menten or the product or substrate inhibition rate expressions. It is therefore useful to discuss which of the two reactor types, tank or tubular, is best suited for these three types of enzyme reactions. Table 1

Table 1. Comparison of Reactor Types

Stirred Tank	High Product Concentration Low Substrate Concentration
Tubular Reactor	High Substrate Low Product

Reaction Type	*Best Reactor Choice*
Michaelis-Menten	Tubular
Product Inhibition	Tubular
Substrate Inhibition	Stirred Tank

summarizes the foregoing comments on relative concentration levels in the two reactor types. Combining this with our knowledge of the enzyme reaction rate expression, we see that the Michaelis-Menten-type reaction will be promoted by high substrate concentration and we can therefore expect the tubular reactor to be more efficient. The product inhibition-type reaction will proceed at a high rate when substrate concentration is low and product concentration is high. The tubular reactor fulfills these conditions. The tank reactor will possess the highest efficiency only for enzyme reaction systems which are inhibited by the substrate. Table 1 summarizes these conclusions.

Although a qualitative understanding of the tank and tubular reactor is extremely useful, a more exact formulation must be undertaken to size the reactor and to determine the influence of the operating parameters. Reactor design equations are based on mass balances for the individual chemical species. The stirred tank reactor is most easily described because the concentrations are uniform. The physical idea behind its mass balance for substrate is as follows:

$$\left[\begin{array}{c}\text{Accumulation}\\ \text{rate of } S\\ \text{within the}\\ \text{reactor}\end{array}\right] = \left[\begin{array}{c}\text{Inflow}\\ \text{rate}\\ \text{of}\\ S\end{array}\right] - \left[\begin{array}{c}\text{Outflow}\\ \text{rate}\\ \text{of}\\ S\end{array}\right] + \left[\begin{array}{c}\text{Reaction}\\ \text{rate}\\ \text{of}\\ S\end{array}\right]$$

For the steady-state operation, after the startup period, the accumulation term is zero. In symbolic form the balance for substrate is:

$$0 = q \cdot S_0 - q \cdot S_1 + r_S\,(S_1, P_1) \cdot V$$

or

$$\tau = \frac{V}{q} = \frac{S_1 - S_0}{r_S(S_1, P_1)}\,.$$

The mean residence time, τ, is given by the ratio of reactor volume, v, and volumetric flow rate, q. The reaction rate is evaluated at the tank outlet conditions and may be a function of S and P, hence $r_S\,(S_1, P_1)$.

The design equation for a tubular reactor is somewhat more difficult to formulate, although based on the same mass balance principles, because the reaction rate varies with position. For an incremental element of reactor volume, the substrate balance is:

$$0 = q \cdot S_n - q \cdot S_{n+1} + r_S\,(S_n, P_n)\,\Delta V,$$

where $\Delta V = A \cdot L$.

Allowing the increments to become small results in the differential form:

$$\frac{dL}{(q/A)} = \frac{dS}{r_S\,(S,P)}$$

Integrating over the entire length gives

$$\tau = \frac{V}{q} = \int_{S_0}^{S_1} \frac{dS}{r_S(S,P)}\,.$$

Thus, the component mass balances lead to design equations for both the tank and tubular reactors. These equations allow the reactor volume requirements to be determined for particular production rates and feed stream conditions. The rate expression, r_S, must be available to make these calculations. Thus, the previously discussed qualitative comparison between types for particular rate expressions is put on a quantitative basis.

Since enzymes are difficult to isolate from their natural source they are expensive substances. For reasons of economy, as well as contamination, for most processes it is desirable to retain the enzyme within reactor. This can be achieved in one of two ways. There exists the possibility of using a membrane-reactor system in which the large enzyme molecule is separated from the product outlet stream by ultrafiltration. Discussion of such reactors follows later. More commonly, the enzyme is immobilized in an insoluble form on a solid material. A variety of materials and methods have been tried and there is much incentive for further research. A stable, active enzyme on an inexpensive support material would make the economic difference for many potentially

attractive enzyme processes. Several reviews[1, 2, 3] serve as an introduction to immobilization techniques.

The performance of reactors whose catalysts are supported on a solid material is generally influenced by the rate of mass transfer. In an immobilized enzyme reactor the substrate must diffuse to the enzyme site before reaction can occur. Following conversion, the product molecule is transported into the bulk liquid phase. Thus, there is a sequence of events which lead from substrate to product:

Substrate in bulk —diffusion→ Substrate on enzyme —reaction→ Product on enzyme —diffusion→ Product in bulk

The overall reaction rate can be influenced or controlled by any one of the individual steps in this sequence. Therefore, in addition to reaction rate data, the design of immobilized enzyme reactors requires information on the diffusion rates. Using this information is often not easy because an exact mathematical description of overall reaction processes in heterogeneous catalysis is very complex and introduces diffusion and mass transfer coefficients which are difficult to estimate. The design engineer must make appropriate estimates of the unknown parameters and rely on simplified models. Uncertainties must often be filled-in with pilot plant data to complete the final design. Although diffusion-reaction phenomena present a complex problem, considerable insight can be obtained by considering simple mathematical descriptions. Thus armed with a qualitative understanding of the immobilized enzyme reaction process, the chemist and engineer are in a position to perform the proper experiments and correctly interpret the data.

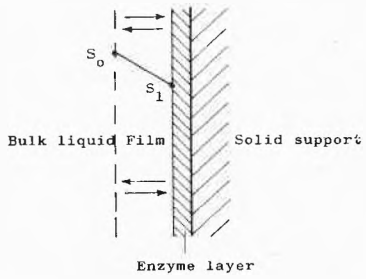


Fig. 5. Liquid film diffusion influence

The simplest example of a supported enzyme diffusion problem is illustrated in Figure 5. Here the substrate is diffusing from the bulk liquid with concentration S_0 to the enzyme surface where the reaction at the surface reduces the substrate concentration to S_E. A concentration difference is thus established between the bulk liquid and the liquid in the immediate vicinity of the reactive enzyme site. After a short time this process reaches steady-state during which:

$$\left[\begin{array}{l}\text{Diffusion rate}\\ \text{of } S \text{ to enzyme}\end{array}\right] = \left[\begin{array}{l}\text{Reaction rate}\\ \text{of } S \text{ to enzyme}\end{array}\right].$$

It is instructive to see the mathematical formulation of this physical statement:

Using Michaelis-Menten kinetics:

$$K_L a\,(S_0 - S_E) = v_m \cdot S/(K_m + S_E).$$

The mass transfer rate is given by the coefficient $K_L a$ times the concentration driving force, $S_0 - S_E$.

For the case when S_E is low, this yields:

$$\text{overall reaction rate} = \left(\frac{K_L a \cdot v_m/K_m}{K_L a + v_m/K_m}\right) \cdot S_0 = \frac{v'_m S_0}{K'_m}.$$

The coefficients v'_m and K'_m are the apparent rate constants.

Actually, they are combined diffusion-reaction constants and are functions of the mass transfer coefficient and the true rate constants as shown.

Two extreme cases exist for this problem: For very high mass transfer rates or very low reaction rates S_E is equal to S_0. The system is then reaction-controlled and the apparent rate constants are equal to the true rate constants. Under this condition the increase in flow stirring rates should have no further influence. For very low mass transfer rates or very high reaction rates the system is diffusion-controlled and S_E is equal to zero. Then the ratio v'_m/K'_m is equal to the mass transfer coefficient $K_L a$. The overall reaction rate will in this case be sensitive to changes in the fluid motion.

Extracting reaction rate constants from experimental data can be a frustrating experience when the role of diffusion is not clearly understood. Results such as shown in Figure 6 are often found when the apparent Michaelis' constant K'_m is a function of the liquid flow rate in a bed of immobilized enzyme catalyst or stirring rate in a tank reactor. The literature indicates that mass diffusion rates often influence supported enzyme reaction rates. Using such data for design purposes is almost impossible unless the mass transfer conditions in the laboratory are well defined.

Small reactor volumes can be achieved by exposing the reacting fluid to large amounts of enzyme. In the case

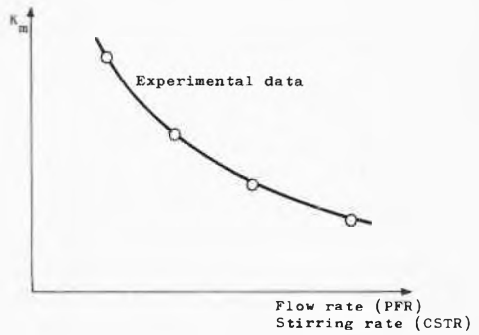


Fig. 6. Kinetic influence of bulk diffusion. Apparent K_m varies with flow rate or stirring rate

of supported enzymes this requires using solid supports with high surface areas on which large amounts of enzyme can be attached or encapsulated. Finely divided and porous solid materials possess high surface areas. Many methods have been used. Enzymes can be encapsulated in polymer beds and fibres as well as adsorbed on porous glass and entrapped in polymers. With such solid supports the catalyst is retained within the external surface of the support. Substrate and product molecules diffuse through the solid to and from the enzyme site. This diffusion process within the solid porous matrix can reasonably be expected to influence the overall reaction rate considerably. Substrates and product molecules of larger size may not be able to penetrate the solid porous structure. Obviously, when selecting the support material care must be taken not to exclude important reaction species from the enzyme site. As with conventional heterogeneous chemical reactors, limited success has been achieved in making a detailed mathematical analysis of enzyme reactions which occur within porous solids. The benefits obtained from making an exact analysis lie in achieving a qualitative understanding of the problem rather than in being able to make exact design calculations.

Consider the situation where an enzyme is adsorbed uniformly within a porous solid as shown in Figure 7. For simplicity, a symmetrical rectangular geometry is assumed. Compared with the liquid film diffusion problem considered earlier, an additional porous solid diffusion process must now be considered. However, the reaction step does not sequentially follow the porous diffusion because active enzyme is present throughout the porous structure. Rather, reaction occurs simultaneously with diffusion as diagrammed below:

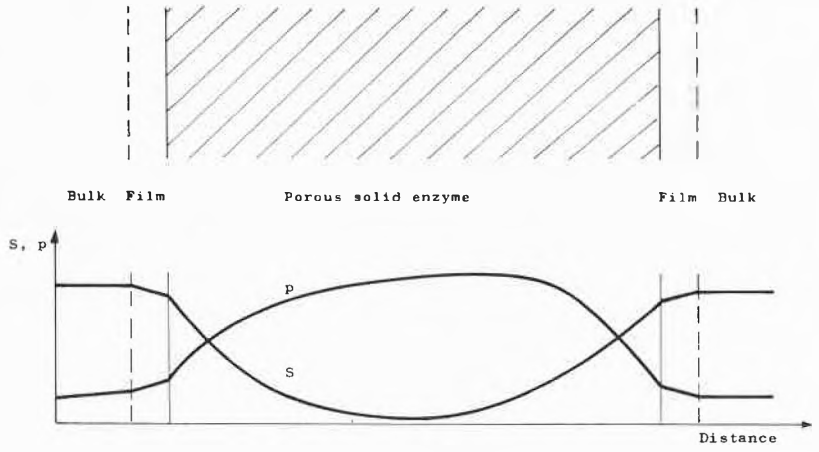
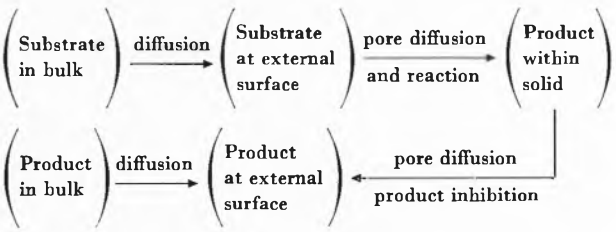


Fig. 7
Concentration in porous enzyme catalysts

The simultaneous diffusion and reaction processes result in concentration gradients of product and substrate. A diffusion equation can be used to approximately describe this problem. Thus, at steady state:

$$D_S \frac{d^2S}{dX^2} + r_S = 0.$$

The boundary conditions are given by:



$$K_L a\,(S_0 - S_E) = D_S \frac{dS}{dX}\bigg|_{\text{external surface}}$$

and, $\frac{dS}{dX} = 0$ at the centre of the solid.

Combined with the reaction rate expression r_S, and a mass balance for P if the reaction exhibits product inhibition, the equation can be solved to give the total rate of reaction within the supported enzyme catalyst. Engineers often use mathematical models to sharpen their thinking about a problem. Although such equation systems can be solved, it is not generally possible to exactly describe an entire reactor taking diffusion within each catalyst particle into account. It can be seen here from the porous diffusion model, without considering the solution, that the important parameters will be the product and substrate diffusivities and the size of the porous support. A very high reaction rate will result in the depletion of the substrate before it reaches the catalyst interior, thus only the outer catalyst volume will be effectively used. This idea can be quantitatively expressed by the "effectiveness factor" concept as normally applied to heterogeneous catalytic reactions. Enzyme systems with low effectiveness factors do not make efficient use of the total available enzyme. Modification of the pore size and catalyst particle size to allow easier access of the substrate to the reaction site will increase the catalyst effectiveness factor. Normally this will also decrease the amount of enzyme which can be loaded on the solid support. The choice of suitable solid supports for enzymes is perhaps the most important unsolved problem in enzyme engineering.

Common reactor types for enzyme catalyzed processes are shown in Figure 8. They include the batch reactor for soluble and immobilized enzymes and the two continuous reactor types, the stirred tank and the packed bed tubular reactor for immobilized enzyme reactions. Consideration of the physical nature of the solid support is necessary in immobilized enzyme reactor design. Catalyst in the tank reactor is present as a suspended solid which is subjected to possible attrition by the mixing device. It must be in a form which can be uniformly suspended by the stirring action.

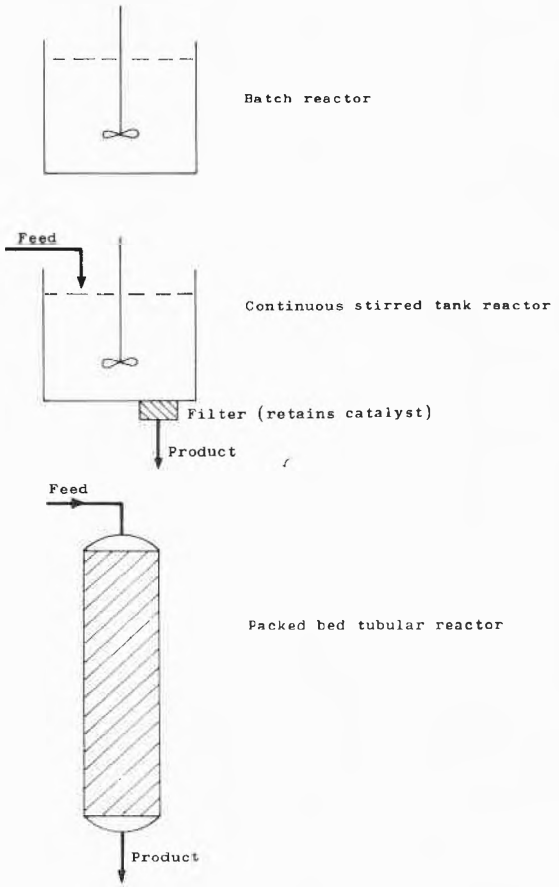


Fig. 8. Common reactor designs for immobilized enzymes

The reactor outlet must be fitted with a filter or some other device to prevent the enzyme from being washed out of the reactor.

The packed-bed enzyme reactor is generally considered to be most practical because of its greater kinetic efficiency with many reactions. Mechanical stirring is not necessary, but the substrate must often be pumped through the reactor. Based on residence time and mass transfer considerations the reactor length and liquid flow rate can be chosen. The nature of the solid support is important in determining the amount of enzyme available for reaction and the pressure drop across the reactor. Many enzyme supports do not possess high mechanical strength and therefore care must be taken in matching them to the reactor. Large forces caused by the weight of a large catalyst bed or high flow rates can cause the beds of softer support materials to compress and block the liquid flow. Mechanical stability, as well as stable activity, must be considered one of the important criteria for a commercially successful immobilized enzyme.

Processes with a soluble enzyme system must be considered with regard to the costs of reclaiming or discarding the enzyme. Soluble enzymes need not be restricted to batch processes. For example, it might be possible to operate a continuous tank or tubular reactor with soluble enzymes which would be more efficient than existing batch processes in which the catalyst is removed by the purification steps and discarded. In terms of future requirements, retaining the expensive enzymes

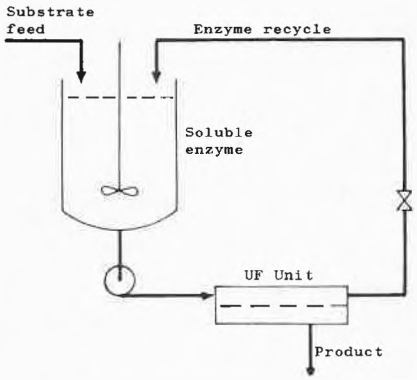


Fig. 9. CSTR Ultra-filtration reactor

appears highly attractive. For this purpose an ultrafiltration membrane system has been proposed, as shown in Figure 9. The idea is based on the fact that enzyme molecules are larger than most substrate and product molecules. A membrane would be selected to perform the required separation. Thus the enzyme could be recycled to the reactor while substrate and product are removed through the membrane. In any actual design the additional membrane and pumping costs would have to be balanced against the savings in enzyme. This reactor design has been evaluated in the laboratory[2].

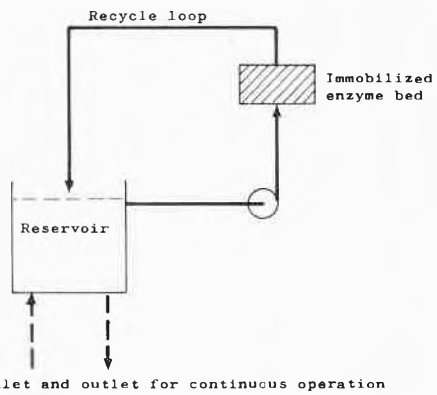


Fig. 10. Differential-bed recycle loop laboratory reactor

Previously it was emphasized that the design of large-scale reactors requires data on the conversion rate at controlled diffusion conditions. The laboratory reactor equipment, shown in Figure 10 and described in a recent paper[5] provides such information. Operating as either a batch or continuous reactor, the liquid is pumped through a short bed of catalyst. With each pass through the bed the extent of conversion is increased by a small amount. It is important that no concentration gradients develop in the catalyst bed, hence the term 'differential bed'. The variable speed pump provides the possibility of controlling the diffusion conditions. High flow rates will eliminate the influence of external diffusion. It is possible with this laboratory device, to investigate the apparent or overall reaction rate under a variety of flow conditions. This information can later be incorporated into design calculations for a full-scale reactor. For immobilized enzyme evaluation studies the external diffusion can be eliminated without subjecting the catalyst to damage by a mixing device. In this way, the influence of the support properties can be investigated free from the masking effects of external diffusion.

Enzyme reaction engineering is a new and exciting field whose success depends on an interdisciplinary effort. The present paper represents a chemical engineer's view of the problems in measuring enzyme reaction kinetics and in designing the enzyme reactor.

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