Scaling Up an Immobilized Enzyme System

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The development of an immobilized enzyme system for commercial application involves a series of decisions and compromises beginning with the choice of enzyme support and ending with the decision on operational mode. Each step is dependent on the other steps, and all the steps influence the overall economics of the final process. Compromises need to be made about the support, method of enzyme attachment, reactor type, kinetic behavior, and operating strategy. Selection of the enzyme carrier and its composition will have a major effect on the *p*H optimum, metal requirements, and overall performance of the immobilized enzyme system. The reactor type and its behavior will also affect performance. For commercial applications the fixed-bed reactor is generally the reactor type of choice. Knowledge of the appropriate kinetics is necessary to achieve the desired product quality and to reduce cost. One important kinetic parameter is reaction velocity under real world conditions. Rate expressions must take into consideration back-mixing and mass transfer limitations, both internal and external to the immobilized enzyme particles. In addition to understanding kinetic behavior, one must devise a proper operating strategy for producing the greatest amount of product at the least cost, while maintaining constant productivity over time. The success of any scale-up will be determined by the final processing cost as compared to that of the alternatives.

BECAUSE OF THEIR ABILITY TO CATALYZE CHEMICAL REACtions at temperatures from approximately 4° to 80°C and at standard pressure, enzymes have been used by man since ancient times. The use of enzymes has gradually been extended into a variety of fields including brewing, food preparation and production, textiles, tanning, and medicine. More recently, immobilized enzymes have been used to improve control, improve product, and reduce cost. Immobilized enzymes are enzymes attached or adsorbed onto water-insoluble matrices such that they can be separated from the liquid medium containing the enzyme substrate and product.

Although Nelson and Griffin (*1*) first reported immobilizing enzymes in 1916, there was little interest in their use until the 1950's and 1960's when studies by Bar-Eli and Katchalski (*2*), Zittle (*3*), MacLaren (*4*), and Mitz and Summaria (*5*) appeared. This activity culminated in the development of the first industrial immobilized enzyme process, which was commercialized by Tanabe Seiyaku Company, Ltd., Osaka, Japan, in 1967 (*6*).

By 1971 when the first Enzyme Engineering Conference was held in Henniker, New Hampshire, a large proportion of the conference was devoted to immobilized enzymes. During these early years there was, understandably, a lack of appreciation for the problems and implications of scaling up such systems. Unfortunately, this lack of understanding has persisted to a significant degree through the intervening years.

At the second Enzyme Engineering Conference in 1973, Havewala and Pitcher (*7*) presented studies on an immobilized glucose isomerase system that for perhaps the first time included an analysis of mass transfer, kinetics, temperature effects on activity, and stability, as well as other considerations of importance required for designing large-scale systems. By 1975 many studies dealing with the engineering of immobilized enzyme systems were appearing. By the late 1970's, immobilized glucose isomerase had become a major commercial success, making possible the production of high-fructose corn syrup.

Today most immobilized enzyme systems in commercial use consist of cells immobilized or entrapped in a water-insoluble matrix, or similarly immobilized cell extracts, or partially purified enzymes. In almost all cases these processes use only one enzyme even if others are present, as in the case of cell extracts. Table 1 lists several commercial and potentially commercial systems that have been developed within recent years.

Because enzymes are catalysts, their cost may be only a fraction of the value of the products they make possible. The enzyme market (approximately $400 million per year) supports several multibillion-dollar industries. Immobilized enzymes constitute only a fraction of the total enzyme market. One difficulty has been that the high initial cost of developing an immobilized enzyme system cannot always be recovered by the enzyme supplier. The strong technical proprietary positions necessary to allow higher valuations of such systems have frequently not occurred.

Assuming a proposed immobilized enzyme system has met the economic and market criteria, what approach should one follow? Developing and scaling up such a system is a large and complex optimization problem wherein a number of interdependent decisions and compromises must be reached to determine the ultimate cost and utility of the system. Since the expenditure of the resources necessary to analyze the situation is usually not justified, certain generalizations and decisions are made on the basis of projections or experience. This article explains the critical factors and the practical criteria that may be used in reaching these decisions. The key areas for examination include the enzyme carrier system, reactor design, operating procedure and strategy, and regulatory considerations if applicable.

Selection of an Enzyme System

In selecting an immobilized enzyme system, three important factors must be considered: (i) source and extent of purification of the enzyme, (ii) form and composition of the carrier, and (iii) type of enzyme attachment.

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Enzyme choice. Until recently the selection of enzyme source was severely limited by the availability of enzymes produced in fermentation at sufficient or potentially sufficient levels to be economically attractive. Recombinant DNA technology has considerably expanded the number of enzymes that can be considered for commercialization.

High specific activity is important for economic production. For large-scale usage, enzymes should be capable of production at the level of grams or tens of grams per liter. The degree of purification required is a cost trade-off, with less purification requiring a larger bulk of immobilized enzyme. Both high- and low-purity approaches have been used. Novo's immobilized glucose isomerase is a cross-linked cell lysate, whereas UOP Inc. and the earlier Corning Glass Works systems depended on more highly purified enzymes and smaller resultant reactor volumes. Cost and reliability of operation are the final arbiters of choice. Occasionally, use of higher purity enzymes may avoid undesirable side reactions caused by the presence of other enzymes or by decreasing residence times.

Enzyme stability is extremely important for immobilized enzyme economics, where long operating life or maximum yield of product per weight of immobilized enzyme is critical. In general, stability has a stronger temperature dependence [typically 10 to 100 kcal (g mol)$^{-1}$] than reaction rate [5 to 20 kcal (g mol)$^{-1}$]. Normally the temperature dependence gives a linear Arrhenius-type plot (logarithm of reaction rate versus reciprocal absolute temperature) (Fig. 1) for the operational half-life. In most cases the highest total productivity is achieved at the lowest temperature. However, the temperature of choice may be dictated by other considerations such as contamination and capital costs, both of which increase at lower operational temperatures.

The *p*H profile of the enzyme of interest in its immobilized form should be one of the first properties investigated since it can influence stability and the choice of support material. The *p*H at which immobilized enzymes operate best may be different from that for the native enzyme. Such *p*H optimum shifts are the rule rather than the exception for immobilized enzymes. An example of a typical *p*H shift for an immobilized enzyme is shown in Fig. 2. The mechanism for the "apparent" *p*H shift has been explained by Goldstein *et al.* (*8*) in their description of microenvironmental effects caused by charged support materials.

The carrier system. If an enzyme carrier is negatively charged, then high concentrations of positively charged ions (H^+) will accumulate at the boundary layer between the support and the surrounding solution. The accumulated H^+ will cause a drop in *p*H below that observed in the bulk solution. Thus the enzyme will be exposed to lower *p*H than measured in bulk solution, thus causing an apparent upward shift in *p*H optimum. The opposite is true for positively charged surfaces. It, therefore, is possible to shift the *p*H of an immobilized enzyme in solutions of low ionic strength by proper selection of the carrier. These effects are decreased in the presence of solutions of high ionic strength.

The influence of kinetics is important in the selection of enzymes. Since most reactions of commercial significance are run at high levels of conversion, kinetic effects such as product inhibition can be severe. For example, the β-galactosidase (lactase) from *Aspergillus oryzae* appears to have less product inhibition than the similar enzyme from *A. niger*. Lactase from yeast also has relatively low product inhibition but poor thermal stability.

The requirement of enzymes for activator ions or ions that increase stability or enzyme activity can sometimes be at least partially met by the incorporation of the ion of interest into the carrier. For example, magnesia incorporated into the porous alumina support developed by Corning for glucose isomerase increased the observed activity by more than 50%.

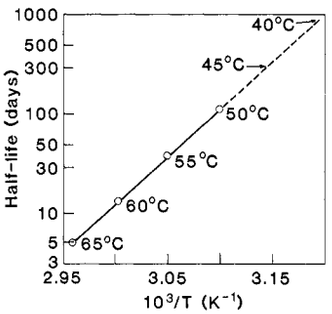

Fig. 1. The effect of reciprocal temperature (degrees Kelvin) on the half-life of glucoamylase covalently coupled to porous silica as determined under continuous operating conditions with 40% enzyme-thinned cornstarch used as a substrate. The actual half-lives were run to completion for 70°, 65°, 60°, and 50°C. The half-life at 45°C was extrapolated after 2400 hours of operation. The maximum operating temperature as determined for most immobilized enzyme systems after 1 hour or less of thermal exposure may indicate conversion rate versus temperature but cannot be used for the determination of final operating temperature. The final operating temperature must be determined by a series of long-term experiments to determine operational half-lives. These data coupled with the rate information allow one to choose that temperature at which greatest productivity is achievable. From (*7*).

In selecting the form and composition of the carrier, one must consider the conditions of operation (*p*H), the enzyme's molecular weight and shape, the reactor design, and the type of enzyme attachment. The overriding factors usually are the cost of the carrier per unit of observed activity and the suitability of the carrier for the reactor design under consideration. Eaton (*9*) has developed a decision tree that aids in the selection of the best possible combination of carrier properties (Table 2).

It is instructive to consider the role of carrier pore structure. The pore size must be large enough to permit easy entry of the enzyme for immobilization and of the substrate for reaction. If the pore diameter is too small, the surface area will not be accessible. Surface area is inversely proportional to pore size; thus, if the pores are too large, the surface area and thus the specific value of the carrier will be low. An example is shown in Fig. 3 for glucoamylase from *A. niger*.

Table 1. Some applications of immobilized enzymes and whole cells. The cell systems use a single enzyme within the killed or attenuated organism. In the whole-cell systems, methods were found to circumvent unwanted side reactions, thus eliminating the need for enzyme isolation and purification. Generally, however, scaling up with a whole cell may require special reactor design and larger reactor volumes because of the lower specific activity of whole cells as opposed to enzymes.

Enzyme	Process	Reference
Glucose isomerase*	Conversion of glucose to fructose	(*19*)
L-Amino-acid oxidase	D-amino acid production	(*20*)
Flavoprotein oxidase	*N*-oxidation of drugs containing hydrazine groups	(*21*)
Δ′-Steroid hydrogenase	Production of prednisolone	(*22*)
Ribonuclease	Nucleotide production for RNA	(*23*)
α-Amylase	Corn syrup production	(*24*)
Glucoamylase	Production of glucose from corn syrup	(*24, 25*)
Invertase	Inversion of sucrose	(*26*)
Penicillin amidase*	6-Aminopenicillinic acid production for semisynthetic penicillins	(*27*)
Aminoacylase	Resolution of DL amino acids	(*28*)
L-Arginine deaminase*	Production of L-citrulline	(*29*)
Fumarase*	Production of L-malic acid	(*30*)
L-Histidine ammonia-lyase*	Production of urocanic acid	(*31*)
Aspartase*	Production of L-aspartic acid	(*32*)

*Whole-cell systems.

This enzyme is oblate-spheroidal with a molecular weight of approximately 100,000. From this information one can estimate the exclusion diameter or the pore diameter at which an enzyme is excluded from entering the pores of the carrier; for glucoamylase this value is 250 Å. Experimentally, the exclusion pore diameter can be determined by the use of liquid chromatography or by coupling the enzyme to supports of different pore diameters. Using the second method, one obtains a "breakpoint" at which the enzyme or substrate is sterically prevented from entering the pores. At this "critical" pore diameter the enzyme activity drops sharply. In the case of glucoamylase, the exclusion diameter is in the region of 300 Å (*9*). Consequently, for this enzyme a support with an average pore diameter above this value was chosen, with 350 Å as the minimum average acceptable value.

Table 2. Some of the important characteristics for an enzyme support material (*9*). These characteristics should be applicable to either an organic or an inorganic material. Decisions regarding these characteristics may in many cases involve a series of trade-offs and compromises when choosing the enzyme support material for final scale-up.

Pore morphology
Ease of immobilization
Chemical desirability of support: effect of *p*H, salt, solvent, and other factors
Convenience of handling
Compression of strength
Enzyme loading
Pressure drop tolerated
Particle size
Particle shape
Flow rates
Substrate solution viscosity
Acceptable half-life
Temperature
*p*H
Salt, solvent, heavy metals
Storage conditions
Economics

Carrier composition. Carrier composition determines the type of enzyme attachment and the durability of the resulting composite. The selection of a chemically inert carrier is important from the viewpoint of both enzyme survival and product purity.

Corrosion or dissolution of the support can shorten the enzyme's operational half-life by enzyme loss or can cause deactivation of the enzyme by inhibition of the catalytic site with soluble corrosion products. Carrier durability can be determined by long-term tests in flow systems at the desired operating conditions. These tests can sometimes be accelerated if the temperature is increased.

If particulate carriers are used, crush resistance and particle size are important. For particles of different size the trade-off is between diffusion limitations with larger particles and problems with smaller particles, such as, higher pressure drop and plugging. Particles in large-scale applications have generally ranged from a fraction of a millimeter to 1 or 2 mm in diameter. Broad pore size distributions and irregular particle shapes decrease reactor bed void volume and dramatically increase pressure drop. For example, at a flow rate of 2.5 liter min^{-1} in a cross-sectional area of 100 cm^2, the pressure drop for a column 3 m long with particles ranging in size from 74 to 125 μm would be 527×10^2 kg m^{-2}. Particles of 125 to 180 μm would yield a pressure drop of less than 351×10^2 kg m^{-2}.

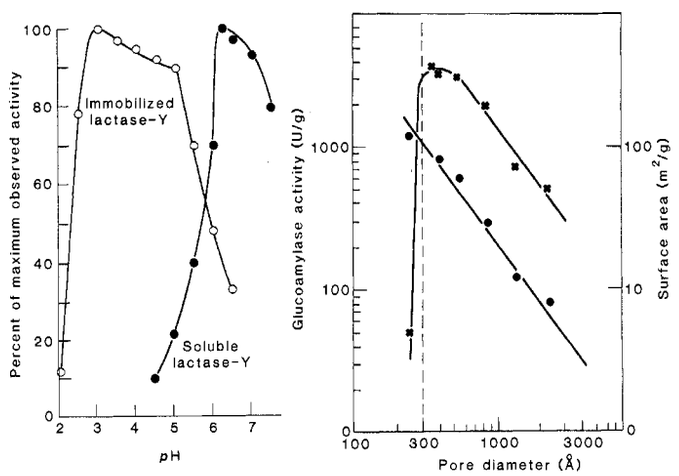

Fig. 2 (left). The *p*H profile of soluble yeast lactase compared with the *p*H profile of the same enzyme covalently coupled to a zirconia-coated glass support. The apparent *p*H optimum of the immobilized enzyme has been shifted 3 *p*H units to the acid side. The immobilized enzyme in this case now shows 80 to 90% of maximum activity at *p*H 4.0; the soluble enzyme is useless at this *p*H. Since this system was designed for the hydrolysis of cottage cheese whey, the *p*H shift brought the enzyme operating *p*H into a useful *p*H range. The choice of carrier can determine the direction and size of any *p*H optimum shift. The ability to control *p*H optimum can allow one to customize an enzyme system to a set of specific feed conditions, as in the case of acid cheese whey. Fig. 3 (right). The relation among glucoamylase activity (+), pore diameter, and surface area (●). The higher the surface area, the higher the enzyme activity per unit volume until the critical pore diameter is exceeded. In the case of glucoamylase, the critical pore diameter was experimentally determined as 300 Å. At this pore diameter the enzyme and substrate can no longer enter the pore (together or singly), thus drastically decreasing the available surface area to the externally available area around the outer portion of the particle.

Reactor Types

Once the enzyme has been characterized and the carrier chosen, one must decide on a reactor design. A number of reactor configurations are available including batch reactors, continuously stirred tank reactors, fluidized-bed reactors, and fixed-bed reactors. Most reactors are combinations or modifications of these types. For example, a series of continuously stirred tank reactors will behave as a plug-flow or fixed-bed reactor. The behavior of a fixed-bed reactor can be made to simulate either a batch or a continuously stirred tank reactor by the simple addition of a recycle loop.

The batch reactor is perhaps the simplest type of reactor. The enzyme or immobilized enzyme is placed in a container with the reactants, and the reaction is allowed to proceed until the desired level of conversion is reached. Since some stirring or agitation of the reaction mixture is usually required, temperature and *p*H control are relatively easy to achieve with rapid response. On the other hand, under these conditions inorganic or other rigid enzyme supports frequently suffer from abrasion, with resulting losses through attrition. Many modifications of the batch reactor have been designed to simplify recovery and reuse of the enzyme composite.

The continuously stirred tank reactor is a stirred tank, operating at steady state. Reactants are fed continuously, and product is withdrawn continuously.

The fluidized-bed reactor, in which the particulate enzyme composite is fluidized by the upward flowing substrate solution, has the advantage of low pressure drop and resistance to plugging. Unfortunately, the fluid velocities required for proper fluidization may lead to residence times insufficient to achieve the desired conversion. Increasing the height of the reactor or using a series of reactors are possible solutions. Reducing particle size or density also decreases the required liquid velocity. Under laboratory conditions, immobi-

lized glucose isomerase (*10*) and immobilized lactase (*11*) fluidized beds duplicate fixed-bed conversion efficiencies at certain bed expansions.

The most widely studied and used reactor for immobilized enzymes in large-scale commercial operations is the fixed-bed reactor. The earliest commercial examples of systems in these reactors were the use of aminoacylase bound to DEAE-Sephadex in a process for resolving DL-amino acids (Tanabe Seiyaku Company) and the use of glucose isomerase Clinton Corn Processing Company (*1*). Later systems that also made use of fixed-bed reactors included immobilized glucose isomerase (Novo Industri A/S) and immobilized lactase (Corning Glass Works). This reactor type will almost certainly continue to dominate large-scale commercial applications because of its high efficiency, ease of operation, and general simplicity. Although there are many forms of the fixed-bed reactor, the most common is a packed bed of particulate material to which enzyme has been coupled. Such packed beds have certain problems. Particulate carrier materials are usually porous to provide greater available surface area for enzyme attachment. Diffusional resistance can limit the apparent activity of the immobilized enzyme if the carrier particles are too large. Unfortunately, the use of very small particles in a packed bed to decrease these problems can result in high pressure drops, bed compaction, and plugging problems.

Thus far in industrial practice, high effectiveness factors have been achieved with acceptable particle size, making packed beds the most efficient and convenient choice for scale-up. Packed beds tend to be less expensive and to have higher enzyme loading per unit reactor volume than the more open reactor designs, such as tubular reactors.

Reactor Behavior

In most industrial applications, high levels of conversion of substrate to product are necessary to achieve the desired product quality and to reduce cost. Knowledge of the appropriate reaction kinetics aids in predicting the performance of certain ideal immobilized enzyme reactors. Actual reactor behavior will only approach, albeit in some cases extremely closely, these theoretical projections.

From a kinetic standpoint, only the three basic types of reactors need be considered: batch reactors, continuously stirred tank or back-mix reactors, and plug-flow reactors. The first two of these reactor types are assumed to be perfectly mixed at all times. The real, as opposed to ideal, performance of continuous reactors generally is somewhere between that of back-mix and that of plug-flow reactors. Under certain conditions, packed-bed reactors can closely approach ideal plug-flow behavior.

Much of the kinetic data reported in the literature for enzyme-catalyzed reactions is initial rate or reaction velocity data. If we take the least complicated case applicable to enzymes, simple irreversible Michaelis-Menten kinetics as an example, the reaction velocity can be written as

$$v = \frac{kES}{K_m + S} \tag{1}$$

where S is the substrate (reactant) concentration, E is the amount of enzyme, k is the rate of enzyme turnover number (product formed), K_m is the Michaelis constant, and $v = -V_s\ (dS/dt)$, where V_s is substrate volume and t is time. Since initial rates are of little interest in the practical world of high conversion levels, one can integrate Eq. 1 from time zero to time t to obtain the following expression for a batch reactor:

$$kEt/V_0 = S_0X - K_m(1 - X) \tag{2}$$

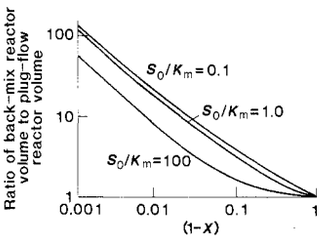

Fig. 4. Ratio of back-mix reactor volume to ideal plug-flow volume required to achieve the same level of conversion. The ratio depends on the initial substrate concentration (S_0), K_m, and the X fraction of substrate converted to product. For low values of S_0/K_m, the kinetics approach first order. At high values of S_0/K_m, the kinetics approach zero order. For zero-order kinetics the ideal back-mix and plug-flow reactors give the same performance.

where V_0 is the initial substrate volume, S_0 is the initial substrate concentration, and the fractional conversion X is equal to $(S_0 - S)/S_0$. In an ideal plug-flow reactor where each volume element of fluid proceeds through the reactor behaving as an infinitesimal batch reactor element without mixing with the adjacent fluid elements, Eq. 2 can be rewritten as:

$$kE/F = S_0X - K_m\ln(1 - X) \tag{3}$$

where V_s/t has become F, the volumetric flow rate.

For the back-mix or stirred-tank reactor, a material balance dictates that

$$v = F(S_0 - S) \tag{4}$$

where S_0 is the feed concentration and S is the concentration in the reactor and at the outlet. If we set the right side of Eq. 1 equal to the right side of Eq. 4, we obtain

$$F(S_0 - S) = \frac{kES}{K_m + S} \tag{5}$$

which after substituting and rearranging becomes

$$\frac{kE}{F} = X\left(\frac{K_m}{1 - X} + S_0\right) \tag{6}$$

Rate equations for other types of kinetic expressions have been described by Pitcher (*12*). By using these equations, one can compare the reactor volumes required for ideal plug-flow and back-mix reactors to achieve the same conversion (Fig. 4). The ratio of reactor volumes for the two cases depends on S_0/K_m and the extent of reaction. For low values of S_0/K_m, the rate expression (Eq. 1) approaches first order and for high values, zero order.

For zero-order kinetics, ideal plug-flow and back-mix reactors give the same performance. In a few cases, such as substrate-inhibited reactions, back-mix reactors are superior. More commonly, because of product inhibition of various types the plug-flow reactor is the more useful of the two.

Integrated rate equations such as Eq. 6 can be visualized as curves of conversion versus residence time normalized to reflect the amount of enzyme present. Normalized residence time can be expressed in various ways, $E_t t/V_s$ for a batch reactor (E_t is total enzyme activity and t is reaction time) and E_t/F for a continuous reactor. A comparison of data for the same immobilized glucose isomerase system from a continuous or a batch reactor is represented in Fig. 5.

This type of curve can be determined experimentally or predicted from the rate equation, with constants calculated from the initial rate data. In practice, it is advisable that one take the empirical approach of determining conversion versus residence time over the full range of interest. The occurrence of side reactions, although minimized by immobilized enzyme systems with low residence times, can still cause deviations from predicted behavior, particularly at high conversions. Diffusion limitations, temperature gradients, and flow nonidealities, such as channeling in packed beds, may cause devi-

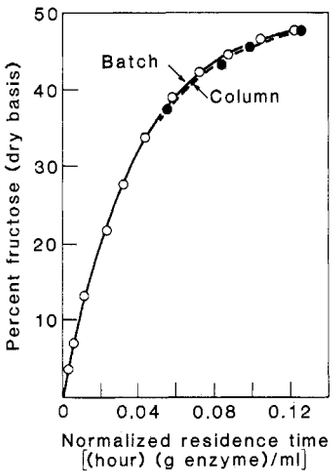

Fig. 5. Conversion versus normalized residence time for a continuous (●--●) and batch reactor (○) for the same immobilized glucose isomerase preparation. For an ideal plug-flow and batch reactor with no mass transfer limitations, conversion and normalized residence time will coincide. Practically, this type of curve can be used along with the observed conversion level to find the corresponding normalized residence time. From this value, if one knows substrate flow rate (or elapsed reaction time and substrate volume for the batch reactor), one can determine the enzymatic activity at the time of observation.

ations from predicted behavior. Many of these factors can be anticipated and their effects predicted.

One of these factors, mass transfer limitation, occurs with sufficient frequency to warrant its discussion here. The effects of diffusion on observed reaction rates are usually separated into two parts, external and internal mass transfer. The effects of external mass transfer, diffusion from the bulk liquid to the carrier surface of the immobilized enzyme, can be estimated in various ways. The condition frequently encountered, laminar liquid flow with very low Reynold's numbers, falls outside the range of many correlations. Pitcher (*12*) has reviewed some of the relevant correlations as well as other approaches to the problem. Satterfield (*13, 14*) has suggested a simple but useful approach consisting of calculating the height of bed required for the necessary mass transfer to occur if film diffusion is assumed to be the rate-controlling step. This method requires only one experimental reactor performance data point (conversion and flow rate) and applies to an integral reactor rather than to just one point within the reactor. The bed height, Z, can be estimated from the following expression:

$$Z = \epsilon \frac{N_{Re}^{2/3} N_{Sc}^{2/3}}{1.09 a_m} \ln \frac{Y_1}{Y_2} \quad (7)$$

where ϵ is bed void fraction, a_m is surface area per unit volume, N_{Re} is Reynold's number, N_{Sc} is Schmidt's number, Y_1 is mole fraction substrate in the feed, and Y_2 is mole fraction substrate in the product. This method was reported by Havewala and Pitcher (*7*), who used it for an immobilized glucose isomerase fixed-bed reactor with Z = 12.8 cm operated at a feed rate of 100 ml hour^{-1} (50% glucose solution) yielding 45% conversion. The Z value calculated (under the assumption that there was film diffusion control) was 0.23 cm, much less than the actual Z. The authors verified the implied absence of significant external mass transfer effects by operating reactors with different Z values at slow rates, which gave the same residence time. If conversion does not change while linear flow is increased by a factor of 2 or more at a constant residence time, then one can eliminate the possibility of significant external mass transfer effects.

Since enzymes are frequently immobilized within porous bodies or retained by semipermeable membranes, limitations from internal mass transfer or pore diffusion are more frequent than from external mass transfer. Simultaneous reaction and diffusion are commonly encountered (*15*). Although rigorous treatment of the problem is beyond the scope of this article, we discuss the basic approaches involved and give examples of practical ways, theoretical and experimental, of evaluating internal diffusion effects.

When a reaction is promoted by an enzyme immobilized within a porous material under steady-state conditions as in a continuous reactor, a substrate concentration gradient is established with the concentration decreasing as the distance from the surface into the porous body increases. The differential equation describing diffusion and reaction in a sphere is

$$\frac{d^2S}{dr^2} + \frac{2}{r}\frac{dS}{dr} = \frac{v_i}{D_{eff}} \quad (8)$$

where r is the radius of the sphere, v_i is the intrinsic reaction rate (usually dependent on substrate concentration), and D_{eff} is the effective diffusivity of the substrate. Only when $v_i = k_v S^m$ (where m and k_v are constants) can an analytical solution to Eq. 8 be found. Cases involving more complicated rate expressions, such as Michaelis-Menten kinetics, must be solved numerically.

The ratio of observed or apparent reaction rate to the rate if no mass transfer limitation existed is called the effectiveness factor (η), which can be calculated from Eq. 8. Various generalized plots relating the effectiveness factor to different kinds of general moduli have been developed. These moduli are of two types, those dependent on the kinetic constants and those based on observed reaction rate data. Bischoff (*16*) expressed η graphically as a function of a general modulus, M, which can be written in terms of Michaelis-Menten kinetic constants where D_{eff} is also constant. For flat plate geometry, this modulus is:

$$M = L\left(\frac{V_{max}}{2K_m D_{eff}}\right)^{1/2}\left(\frac{S}{K_m + S}\right)\left[\frac{S}{K_m} - \ln\left(1 + \frac{S}{K_m}\right)\right]^{1/2} \quad (9)$$

where L is plate thickness and V_{max} is maximum velocity; for M greater than 2, $\eta = 1/M$. The effectiveness factor plot based on this modulus is shown in Fig. 6. A similar approach (*12*) based on a modulus that incorporates observed reaction rates can be used. Moo-Young and Kobayashi (*17*) have developed numerical solutions for more complex product and substrate inhibition kinetics. The complicated and lengthy moduli calculation undoubtedly discourages the use of their solutions.

Probably the largest source of error for any of the moduli is the effective diffusivity, which is difficult to measure or estimate. There are several practical ways of estimating the effects of pore diffusion to avoid this problem. One method is to decrease particle size or membrane thickness until no further increase in reaction rate is observed. This final rate can then be assumed to be the intrinsic rate and can be used to calculate the appropriate effectiveness factors. Care must be taken to avoid artifacts in these experiments. Crushing particles to reduce their size may affect the observed activity by plugging or altering pore structure. On the other hand, immobilization of enzymes on different-sized particles should be done in separate batches to avoid preferential enzyme uptake by the smaller particles.

Another practical way to estimate the effect of internal diffusion is to compare the dependence of reaction rate on temperature for the immobilized enzyme as compared to the soluble enzyme. If an Arrhenius plot for the immobilized enzyme gives a straight line over the range of interest with a slope equal to that for the soluble enzyme, it normally implies that no resistance to pore diffusion exists. Pore diffusion limitations will usually cause a decrease in slope at higher temperatures in a plot of this kind. This slope will approach one-half that at lower temperatures since the apparent activation energy approaches the (relatively small) arithmetic mean of the diffusion and chemical reaction activation energies.

Pore diffusion effects can be reduced in several ways. Enzyme loading should be optimized, taking into consideration the trade-off

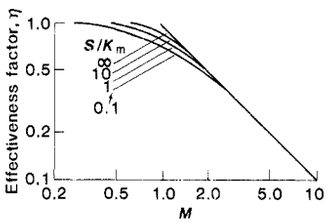

Fig. 6. Effectiveness factor for Michaelis-Menten kinetics: A general modulus M (Eq. 9) written in terms of Michaelis-Menten kinetic constants assuming that substrate diffusivity does not vary with concentration. The effectiveness factor describes the ratio of observed apparent rate to the rate with no diffusion limitation. For an immobilized enzyme system, the value should be near unity for efficient operation.

between efficient enzyme use at high loading and larger carriers and immobilization cost at low enzyme loadings. Diffusion problems can also be alleviated by decreasing particle size or by immobilizing the enzyme in a layer near the particle external surface. In real reactors ideal plug-flow behavior is never achieved since some degree of back-mixing or dispersion inevitably occurs. Correlations for dispersion coefficients have been developed to predict back-mixing effects. Tracer studies can be used to determine dispersion experimentally. Unfortunately, channeling and other irregularities in liquid-carrier contacting, not readily amenable to accurate analysis, are frequently more important than back-mixing.

Operating Strategy

Pitcher (*12, 15*) has discussed the operating strategy for immobilized enzyme reactors, a question of economic importance. The ultimate goal is to minimize total processing cost. In practical terms, this usually means maximizing the total product that can be produced by a given amount of immobilized enzyme composite. The total production P of a reactor during time t_p can be related to the feed rate F by the equation:

$$P_t = \int_0^{t_p} F dt \tag{10}$$

For the most common case, operation at constant conversion with exponential decay gives

$$P_t = \int_0^{t_p} F_i e^{-(\ln 2)t/t_{1/2}} dt \tag{11}$$

where F_i is initial feed rate and $t_{1/2}$ is the immobilized enzyme half-life. When this expression is integrated, one obtains

$$P = F_i t_{1/2} \left[1 - e^{-(\ln 2)t_p/t_{1/2}} \right] \tag{12}$$

Such isothermal operation at constant conversion requires decreasing flow rate to maintain the conversion level as enzyme activity falls. This normally unacceptable change in production rate is usually dealt with by the use of a multiple-reactor system. Start-up or reloading time for each reactor is staggered, with the reactors operated in series or parallel. The number of reactors required to maintain the production rate within a given range is a function of the number of half-lives for which the enzyme is used. Figure 7 shows an example. Most commercial glucose isomerase systems are operated in this manner.

Another operating strategy is to raise the temperature to compensate for activity loss at a rate to maintain the original production rate and conversion levels. This procedure has been used commercially with immobilized lactase where half-lives are long and multiple-column systems are an unnecessary complication. Other operating strategies may be optimal for other, less frequently encountered circumstances.

Operating costs. In the development of immobilized enzyme systems, preliminary cost estimates are a useful tool for determining economic feasibility and for identifying problem areas in potential systems. The cost can be broken down into several components. The first is the cost of the immobilized enzyme, which includes carrier, enzyme, and immobilization costs plus labor, equipment, capital, and materials. This composite cost can be used with performance data to calculate the processing cost attributable to the composite. The second cost component is labor, including fringe benefits, supervision, and other overhead charges. The third cost component, equipment, appears as a depreciation charge. Taxes, insurance, maintenance, and other miscellaneous items are commonly estimated as a percentage of capital and along with depreciation may in some cases exceed 20% of capital annually. Raw material cost must be added to these processing costs to obtain the product cost. Provision must be made for losses at each processing step.

Perhaps the least appreciated costs with the highest impact are those associated with preparing feedstock and purifying or concentrating product. The costs of other parts of a total manufacturing process can dwarf the cost of the immobilized enzyme system. For example, in whey hydrolysis the concentration of the hydrolyzed lactose is a much more expensive process than the hydrolysis. Even concentration and transportation of the feedstock in this case can exceed the enzymatic processing costs.

Although many of the characteristics of immobilized enzymes and reactors have been discussed individually, they are not independent. Enzyme kinetics, substrate concentration, pH, activators, temperature, and carrier size and shape may all affect the rate at which an immobilized enzyme catalyzes a reaction.

Both enzyme loading, the amount of enzyme immobilized per unit volume or weight of support material, and the cost of the support affect the total processing cost. For inexpensive enzymes, high loading is essential to reduce the cost of support material. Reactor size is also reduced when the amount of composite decreases. However, if the enzyme is expensive, its efficient utilization is important. The greater the amount of enzyme immobilized on a given amount of carrier, the less efficiently that enzyme is used. The percentage of activity of a soluble enzyme consumed in immobilization that is later observed as activity of the immobilized enzyme decreases. Enzyme of higher purity can increase this coupling efficiency for a given activity level in some cases.

During scale-up to pilot plant and commercial plant levels, several considerations become increasingly important relative to their significance at the bench level. Feed composition frequently is a critical variable in system performance with examples of half-lives ranging from 2 to over 100 days for immobilized lactase operated at the same temperature with a variety of feeds. Laboratory results with

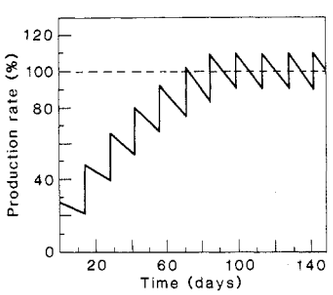

Fig. 7. Multiple column system production rate. Operating isothermally at constant conversion would require decreasing flow rates or production rates to maintain constant conversion as the enzyme activity decreases versus time. A common method of maintaining constant conversion and productivity is to use a multiple column approach. Start-up and reloading times for individual reactors are staggered with reactors operating either in parallel or series. The figure shows the production rate (as a percentage of desired rate) as a function of time from start-up for a case where the rate from maximum to minimum production has been set at 0.82, the number of columns in the system is seven, and the number of half-lives used is two. This system operates at constant temperature.

reagent grade feedstocks are frequently not repeatable when less pure commercial materials are used. Removal of salts, proteins, or other contaminants is sometimes justifiable to prolong the operating life of the enzyme. In the case of immobilized lactase, where the sugar concentrations are 4 to 15%, microbial contamination can severely affect performance and can necessitate periodic cleaning. Other systems such as glucose isomerase, where sugar levels are in the 50% range with 60°C temperatures, have little or no microbial contamination.

Other problems encountered in commercial operations include enzyme composite bed compression, which leads to unacceptably high pressure drop and further bed compression. Microbial contamination can aggravate this problem. Carrier attrition upon handling may generate fines that are either washed away, which decreases the total available activity, or retained in the reactor, which causes increased pressure drop.

Since in many cases the immobilized enzyme composite must be used for a relatively long period (several months or more), fail-safe controls for temperature, *p*H, and other parameters that could result in catastrophic activity loss should be installed. Failure to consider the immobilized enzyme system in the context of the overall process may lead to unrealistic expectations for the immobilized enzymes.

Regulatory Considerations

Another important consideration when developing immobilized enzyme systems for certain uses, such as in the production of food or pharmaceuticals, is regulatory status (*18*). In the United States, the Food and Drug Administration (FDA) (most other developed countries have a similar agency) regulates the use of immobilized enzymes in the production of food. The regulations in the United States (21 Code Federal Regulations 17,360) require that the fixing agents (term used by the Federal Drug Administration) for immobilization consist of substances generally recognized as safe (GRAS) in food or (with two exceptions) that the fixed enzyme preparation be washed to remove residues of fixing materials. The only two fixing agents specifically approved for use for the preparation of immobilized glucose isomerase for the production of high-fructose corn syrup are diethylaminoethyl cellulose and glutaraldehyde.

At least four petitions for GRAS affirmation were pending with FDA at the end of 1985. Corning Glass Works is seeking GRAS affirmation (petitition filed 9 March 1981) of a composite prepared by covalently bonding β-galactosidase from *A. niger* or other approved organisms to a porous ceramic support via the linking agents γ-aminopropyltriethoxysilane and glutaraldehyde for producing low-lactose whey. Amerace has submitted a petition (18 June 1984) to affirm GRAS status of an insolubilized lactase preparation derived from *A. oryzae* and immobilized with glutaraldehyde and polyethylenimine for use in producing lactose, hydrolyzed whey, lactose-hydrolyzed whey permeate, and lactose-hydrolyzed milk permeate. The FDA ruled as inadequate a process and enzymes described in UOP Inc.'s petition (23 November 1981) involving high-fructose corn syrup prepared from corn syrup glucose by the action of a glucose isomerase preparation derived from *Streptomyces olivochromogenus* and immobilized with polyethylenimine cross-linked with glutaraldehyde. The same fate has befallen Miles Laboratories' petition (25 June 1984) involving the use of *Flavobacterium arborescens* cells (nonviable), polyethylenimine-ethylene dichloride polymer, chitosan, and glutaraldehyde reaction product for use in the production of high-fructose corn syrup. FDA is also in the process, as of early 1986, of formulating GRAS regulations covering a wide range of enzymes, most of which are already in use and some of which could be used in immobilized form if the criteria for fixing agents, discussed above, are met.

Many of the cross-linking or fixing agents that have been reported in the literature as useful for enzyme immobilization are not approved for use in food. For some of these, sufficient safety data may be lacking; others are known to be highly toxic. One problem in establishing safety is that cross-linking agents are by nature highly reactive and may possibly be toxic. On the other hand, immobilized enzyme composites, if reacted to completion and well washed, should contain little reactive material and, in many cases, pose no hazard. Nevertheless, to meet the food safety requirements, residual levels of reactive coupling material should be determined and a 90-day feeding study with the enzyme composite should be carried out to demonstrate safety.

Future Developments

Little progress is likely to come from innovations in reactor design except in unusual applications. Enzyme immobilization has been progressing toward higher enzyme loading, better durability and rigidity of supports, and lower cost with potential for improvement. Improvements in cofactor regeneration also appear to be ongoing.

Probably the area that will lead to the greatest advances is enzymology and new applications of enzymes. Recombinant DNA technology brings many more enzymes under consideration for production at commercially interesting levels. Enzyme engineering, modification of enzymes in a controlled fashion, holds even greater promise for designing enzymes for specific uses.

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