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CYCLE TIME AND CARRIER LIFE IN IMMOBILIZED-GLUCOAMYLASE REACTORS

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The activity of immobilized enzymes decays with time, and the capacity of a carrier will decrease with repeated regeneration. Relations between production cost and these factors are shown, and demonstrated with data on glucoamylase immobilized on porous glass. Optimum design calls for very low temperature and for cycle times several years long. A practical design may be made by limiting cycle time to an upper limit and calculating the temperature for which this time is optimum. In this case, reagents and carrier are the most important costs, even with an expensive enzyme.

INTRODUCTION

Immobilization of an enzyme makes it possible to use it in a continuous reactor, thus reducing the cost of the catalysis. The penalty for immobilization arises from the cost of the carrier, the reagents used, and any losses caused by modification of the enzyme. Immobilization generally extends the life of an enzyme, but eventually a reactor must be removed from operation and fresh enzyme put on the carrier. The activity and stability of immobilized enzymes, which are needed to make these decisions, have been measured in many studies (1–8). An important economic decision that must be made is how long to operate before regenerating. Only one published paper, by Pitcher and Weetall (9), has addressed this problem.

Carriers, in turn, do not have infinite life, but slowly lose their capacity for enzyme during use and regeneration, and eventually must be replaced. Unfortunately, there are no data in the literature on degradation of a carrier, let alone its economic management. The object of the work reported here was to find relations involving cycle time and carrier life in immobilized enzyme reactors, and to demonstrate the impact of the parameters, using our data on decay of activity of glucoamylase bound to porous glass, and using some speculations on the decay of carrier capacity.

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THEORY

A reactor should be designed on the basis of optimum values of cycle time (the time of operation before cleaning the carrier surface and adding fresh enzyme), and the number of cycles a carrier is used before being discarded. These issues are addressed in this section.

The total cost of the operation is calculated as the sum of costs of enzyme, carrier, immobilization reagents, and reactor shell. A number of important conclusions can be drawn from just these costs, without taking account of the other costs of operation (e.g., labor, power, instrumentation, taxes).

The reactor consists of several modules on-stream in series at one time, with one additional module being regenerated. The flow rate through the train is held constant, and periodically one module is removed from the train for regeneration and a fresh one is substituted. The module having the lowest activity should be located at the downstream end of the train, where the concentration of reactant is lowest, to minimize diffusional problems (which are ignored below).

Carrier capacity will be assumed to be lost only during regeneration, such as by strong base or high temperature. It might also be lost during the on-stream period, caused perhaps by polymers that hydrolyze away or by the attrition of a fluidized bed, but this occurrence leads to similar conclusions and will not be shown.

If the carrier actually disappears, then it might be possible to pour fresh carrier into a module at the time of each regeneration to bring it back to its original capacity for enzyme. This case, called below the "recovered carrier" case, leads to some simple results involving just one economic parameter. A more realistic mode of operation is to operate each module containing only the carrier originally charged (or what is left of it) for many enzyme regeneration steps, and then throw away the remains of the carrier and recharge the module with fresh carrier. This case, called the "dissipated carrier" case, results in a more complicated analysis involving two economic parameters.

The Recovered Carrier

Carrier is presumed to disappear during the removal of the spent protein material from its surface, and a quantity of fresh carrier is poured into the module each time new enzyme is bound, to bring the initial activity of the module to the original value. The total cost of the operation is given by:

$$C = (C_1/\theta + C_2)V \tag{1}$$

where

$$C_1 = [10^3 C_E V_0 + C_R R + C_S(1-L)]hN \tag{2}$$

and

$$C_2 = C_D 10 Dp(N+1)/A \tag{3}$$

in which C is the total cost per year, \$; C_E is the cost of enzyme, \$/unit of initial immobilized activity; C_R is the cost of reagents, \$/g; C_S is the cost of carrier, \$/liter; C_D is the cost of reactor shell, \$/m; Dp is the fractional rate of depreciation, yr^{-1}; R is the amount of reagent needed, g/liter reactor volume; V_0 is the initial enzyme activity loading, units/ml reactor volume; h is the days of operation in 1 year; θ is the cycle time, days of operation before regeneration; L is the fraction of carrier capacity remaining after each regeneration; A is the empty cross section of the reactor, cm^2; N is the number of modules on-stream at one time; and V is the volume of one module, liters. C_1 and C_2 are useful cost functions that are independent of cycle time and temperature. The "unit" of activity is 1 μmol product/min.

The cost of the reactor shell is calculated here by picking a value of cross-sectional area that is reasonable for the range of conditions covered and allowing the length to change to provide the volume needed. This method has the effect of making this cost proportional to the volume, which simplifies the resulting expressions and allows some important generalizations to be more easily made.

The production in one cycle per module is:

$$P_\theta = \mathrm{M} \int_0^\theta F(S_1 - S_2)\, \mathrm{d}t \tag{4}$$

in which P_θ is the production in 1 cycle for 1 module, g; F is the flow rate, liters/min; M is the min/day, 1440; S_1 and S_2 are the inlet and outlet substrate concentration for this module, g/liter; and t is the time, days. The total production per year, P, is Nh/θ times this figure.

The kinetics of the reaction is taken to be zero-order to allow some generalizations to be easily shown. In fact, zero-order is reasonable for many hydrolytic reactions in which the feed concentration of substrate is high and the concentration does not approach K_m, as it does not in the example that follows. Plug flow is assumed for the packed-bed reactor, giving the reactor equation:

$$F(S_1 - S_2) = KV_{max}V \tag{5}$$

in which V_{max} is the reactor activity, units/ml reactor volume and K is the conversion factor, 0.18 for glucose production. The zero-order rate

constant, V_{max}, is assumed to decay in a first-order fashion:

$$V_{max} = V_0 \exp(-t/\tau) \tag{6}$$

in which τ is the time constant, days. Production per year is then:

$$P = NVV_0hK\mathrm{M}[1-\exp(-\theta/\tau)]\tau/\theta \tag{7}$$

This is used to eliminate V from Eq. (1), yielding:

$$C = \frac{P}{NhK\mathrm{M}} \frac{1}{V_0\tau} \frac{(C_1+C_2\theta)}{[1-\exp(-\theta/\tau)]} \tag{8}$$

This is the starting point for the calculation of best cycle time and the effects of temperature.

Optimum cycle time, from the derivative of Eq. (8) with respect to cycle time, is given by:

$$r = \exp(\theta/\tau) - \theta/\tau - 1 \tag{9}$$

in which the cost ratio r is defined as $(C_1/C_2)\tau$. The results that follow for the "recovered carrier" case can be characterized by this single economic parameter. The value of r for immobilized-enzyme reactors will be high, meaning that the cost of enzyme, reagents, and supports will be larger than the cost of reactor housing. Figure 1 is a plot of Eq. (9). Combining Eqs. (8) and (9) gives the minimum cost when the optimum cycle time is used:

$$C = \frac{PC_2}{NhK\mathrm{M}V_0} \exp(\theta/\tau) \tag{10}$$

The effects of temperature are seen in changes in V_0 and τ:

$$\frac{\mathrm{d}V_0}{\mathrm{d}T} = V_0 \frac{E_V}{RT^2} \tag{11}$$

$$\frac{\mathrm{d}\tau}{\mathrm{d}T} = -\tau \frac{E_\tau}{RT^2} \tag{12}$$

in which E_V and E_τ are the activation energies in the Arrhenius equation. The effect of temperature on the optimum cycle time is found by taking the derivative of Eq. (9):

$$\frac{\mathrm{d}\theta}{\mathrm{d}T} = \tau\left[\frac{r}{\exp(\theta/\tau)-1} - \frac{\theta}{\tau}\right]\frac{E_\tau}{RT^2} \tag{13}$$

It can be shown that the bracketed term is always negative, so the optimum cycle time goes down as temperature increases.

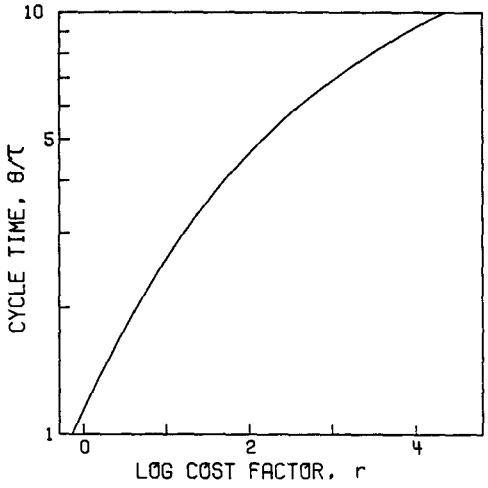

FIG. 1. Optimum cycle time as a function of costs for the "recovered carrier" case. Cost factor r is $C_1/C_2\tau$. C_2 contains the reactor-housing cost; the other costs are in C_1. For immobilized enzyme reactors, r, and thus cycle time, will be large.

The effect of temperature on total cost is found by taking the derivative of Eq. (10):

$$\frac{dC}{dT} = \frac{C}{RT^2}\left[\frac{r}{(\exp(\theta/\tau)-1)}E_\tau - E_V\right] \tag{14}$$

For immobilized-enzyme reactors the value of r at customary operating temperatures will be high, and the coefficient of the first term will usually be close to unity. Since the first activation energy will almost always be larger than the second, cost will go up as temperature goes up, and the best temperature of operation will be below the customary temperatures.

The Dissipated Carrier

In this case, carrier capacity is reduced during the removal of spent protein from its surface, but unlike the previous case, the module is not disturbed until after n regenerations, and the carrier is then thrown away. After each regeneration, the capacity of the carrier will be L time what it was before. At the beginning of the ith cycle, the enzyme activity in a module is L^{i-1} times what it was when the carrier was fresh, so that Eq. (6)

is replaced by:

$$V_{max} = V_0 L^{i-1} \exp(-t/\tau) \tag{15}$$

Production per cycle per module will be the same as before multiplied by L^{i-1}, and this must be summed over the n cycles for which the module is operated before changing carrier, which leads to the factor:

$$\sum_{i=1}^{n} L^{i-1} = \frac{(1-L^n)}{(1-L)} \tag{16}$$

Finally, the production per n cycles is multiplied by $Nh/n\theta$ to give the total annual production:

$$P = NVV_0\tau hK\mathrm{M}\frac{(1-L^n)(1-\exp(-\theta/\tau))}{(1-L)n\theta} \tag{17}$$

Total cost in this case is:

$$C = \left[\frac{C_1}{n\theta}\frac{(1-L^n)}{(1-L)} + \frac{C_2}{n\theta} + C_3\right] V \tag{18}$$

where

$$C_1 = [10^3 C_E V_0 + C_R R]hN$$

$$C_2 = C_S hN$$

$$C_3 = C_D 10Dp(N+1)/A$$

Eliminating V by means of Eq. (17):

$$C = \frac{P}{NK\mathrm{M}h}\frac{1}{V_0\tau}\frac{[C_1 + C_2[(1-L)/(1-L^n)] + C_3[(1-L)/(1-L^n)]n\theta]}{1-\exp(-\theta/\tau)} \tag{19}$$

This is the starting point for the calculation of best cycle time and number of cycles per batch of carrier.

Optimum number of cycles is obtained from the derivative of Eq. (19) with respect to n:

$$\frac{r_2\tau}{\theta} = \frac{L^{-n}-1}{(-\ln L)} - n \tag{20}$$

where $r_2 = C_2/C_3\tau$. When L approaches unity:

$$\frac{r_2\tau}{\theta} = n^2(1-L)/2 \tag{21}$$

Equation (20) is plotted in Fig. 2, modified to take advantage of the

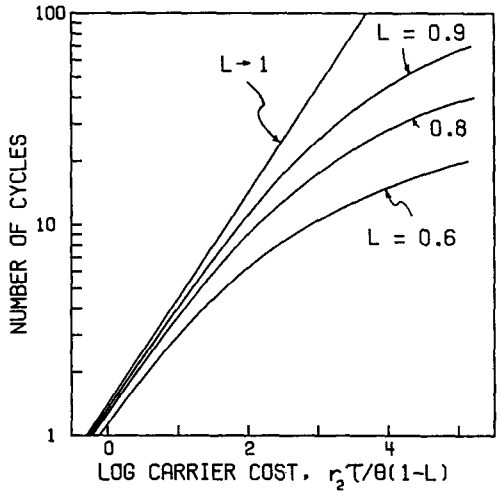

FIG. 2. Effect of carrier cost and carrier loss on the optimum number of cycles run before discarding the carrier in the "dissipated carrier" case. The term $(1-L)$ is the fractional carrier capacity lost on each regeneration. The function shown on the abscissa is used to bring the curves close together.

asymptotic relation given by Eq. (21). It gives the optimum number of cycles, n, in terms of the optimum cycle time.

The derivative of Eq. (19) with respect to cycle time is:

$$r_1\frac{(1-L^n)}{(1-L)}+r_2=\left[\exp(\theta/\tau)-\frac{\theta}{\tau}-1\right]n \tag{22}$$

where $r_1 = C_1/C_3\tau$.

The results that follow for the "dissipated carrier" case can be characterized by these two economic parameters, r_1 and r_2. Their values for immobilized enzyme reactors will be high, meaning that the cost of enzyme and reagents in the first case, and support in the second, are greater than the cost of reactor housing.

Figure 3 shows solutions of Eqs. (20) and (22) for the case $L = 0.8$. At high enzyme and/or reagent cost (high r_1), carrier cost has very little influence on the cycle time, but does influence the number of cycles, as shown by Fig. 2. At low cost of enzyme and reagent, on the other hand, cycle time is determined solely by support cost. The value of L has no effect at high values of r_1, but does influence the point of deviation from the common trend at the low end.

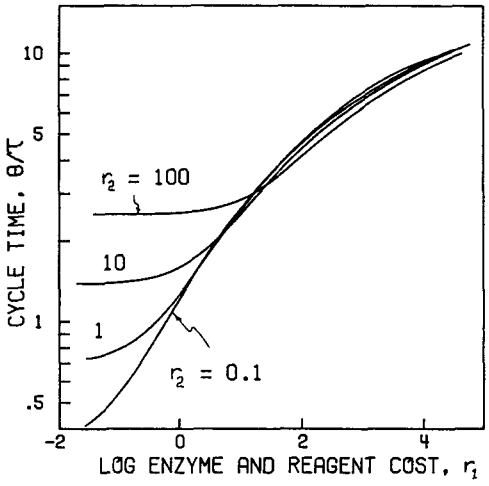

FIG. 3. Effect of costs on optimum cycle time in the "dissipated carrier" for the case $L = 0.8$. Cost factor r_2 contains the ratio of cost of carrier to cost of reactor housing. Cost factor r_1 contains the ratio of costs of enzyme and reagents to cost of reactor housing. At high enzyme or reagent cost, carrier cost has little effect on cycle time.

Combining Eqs. (19) and (22) gives Eq. (23), which is the minimum cost when the optimum values of cycle time and number of cycles from Eqs. (20) and (22) are used:

$$C = \frac{P}{NKMh} \frac{C_3}{V_0} \frac{(1-L)}{(1-L^n)} n \exp(\theta/\tau) \tag{23}$$

The equations developed for these two modes of handling carrier losses will now be demonstrated using our data on glucoamylase immobilized to porous glass.

MATERIALS AND METHODS

General

Glucoamylase (α-1,4-glucan glucohydrolase, E.C. 3.2.1.3) was obtained from Sigma Chemical Company in the form of a crude preparation containing an inert extender. The enzyme was solubilized by suspending the mixture in 0.05 M acetate buffer, pH 4.25. The inert extender was then removed by filtration.

The substrate chosen for the assay of the enzyme was maltose. While maltose is a poorer substrate for the enzyme than higher oligosaccharides, it is simpler to work with. The grade II maltose was obtained from Sigma Chemical Company.

The glucose produced by the hydrolysis of the maltose catalyzed by the enzyme was analyzed by glucose oxidase, peroxidase, and *o*-dianisidine, all purchased from Sigma.

Porous Glass

Alkylamine glass (55-nm pore size) was obtained from Corning Glass Works under the brand name Encor. A 4-ml sample of the nominal 40/80 mesh glass was activated with 2.5% glutaraldehyde solution buffered to pH 7.0 with 0.1 M phosphate. The total volume of about 8 ml was slowly stirred at room temperature for 1 h, and then washed with 20 vol distilled water to remove excess glutaraldehyde. The glass was next suspended in 0.1 M pH 7.0 phosphate buffer containing 900 mg protein, and slowly stirred at ice-bath temperature for 1 h. At the end of the attachment, 20–30 vol of substrate buffered to pH 4.25 with 0.05 M acetate was used to wash out excess enzyme.

At this point, the batch was divided into three separate portions to be treated differently, each packed into a tubular reactor about 5 cm tall and having a cross-sectional area of 0.256 cm^2. The first of the three reactors was put into continuous use at 40°C, and a series of studies was made to determine the effect of flow rate and substrate concentration. The second reactor was exposed to 6 M urea at room temperature for various lengths of time, and the activity of the column was determined after each. The third reactor was tested for thermal stability by operating it continuously at 60°C. The operation of these columns consisted of flowing 475 mg/ml of substrate solution through them at a slow rate at the prescribed temperature. To determine the activity, the flow rate was increased, and the conversion yielded by the reactor was measured.

RESULTS

In order to be assured that the productivity exhibited by a reactor is the maximum, one must eliminate the effects of bulk and pore diffusion and saturate the enzyme. To find the proper conditions, substrate concentration fed to the plug-flow reactor was varied from about 10 times K_m to 100 times K_m ($K_m = 0.37$ mg/ml). Two superficial velocities were used, 7.8 and 1.95 cm/min.

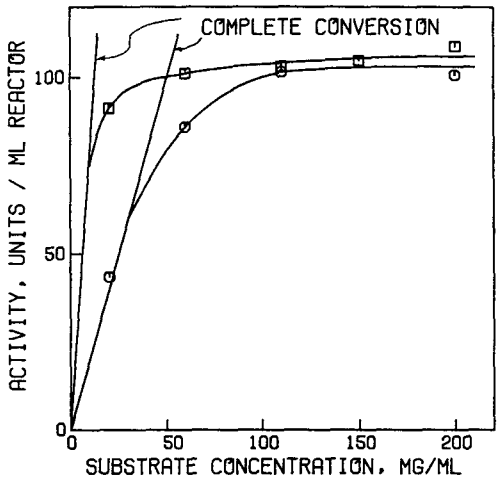

FIG. 4. Influence of substrate concentration and flow rate on activity in a plug-flow reactor. Superficial velocities were 7.8 (□) and 1.95 cm/min (○). Feed was maltose in 0.05 M acetate buffer at pH 4.25. Activity reaches an upper limit at about 100 mg/ml, showing no effect of diffusion above that concentration. Straight-line limits at the low end show expected activity if all the substrate were reacted. K_m is very low (0.37 mg/ml), so the expected behavior is very close to the intersecting straight lines. The gradual curving deviations from these lines show diffusional resistance.

The results are shown in Fig. 4. The two straight lines with high slope near the origin show the activity to be expected if all the substrate were consumed at each flow rate, and each curve approaches this limit at the low end. At the high end, it seems clear that each curve approaches a constant value, indicating that there is no diffusional resistance. Between these two straight-line portions, there is a transition zone in which the curves depart from these linear limits. Since no concentration was less than 10 times K_m, the enzyme must have been saturated throughout, and this departure indicates diffusional limitation. To avoid errors caused by diffusion, all subsequent data were collected at substrate concentrations at least twice that at which the upper straight line is reached.

The effect of urea on the immobilized enzyme is shown in Fig. 5. The reactor lost activity for about the first hour of exposure, but retained 80% of the initial activity thereafter.

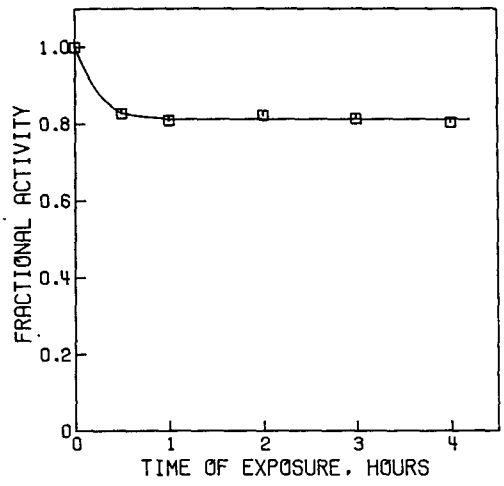

FIG. 5. Effect of 6 M urea on glucoamylase immobilized on porous glass. The immobilized enzyme was soaked in urea at room temperature for a period, washed, and the activity measured.

Stability of the immobilized glucoamylase is shown in Figs. 6 and 7. Results are summarized in Table 1. The half-life of the immbolized enzyme at 60°C is 11 times that of the soluble enzyme.

Glucoamylase has been immobilized many times (4,7,8,10–14), but few results are directly comparable to ours. Pitcher and Weetall have reported information on glucoamylase from a different source on a different batch of porous glass, using liquified starch as feed (9). Activities cannot be compared, because of the difference in substrates, but stabilities can. From their data, we calculate an activation energy for decay of activity of 41 kcal, very close to our value of 39 kcal. However, at any one temperature, they attain a

TABLE 1. **Influence of Temperature on Properties of the Continuous Reactor**

	Initial activity[a]	Half-life
At 40°C	101 units/ml	150 days
At 60°C	370 units/ml	85 h
Activation energy	reaction: 14 kcal/mol	deactivation: 39 kcal/mol

[a]The "unit" of activity is 1 μmol product/min.

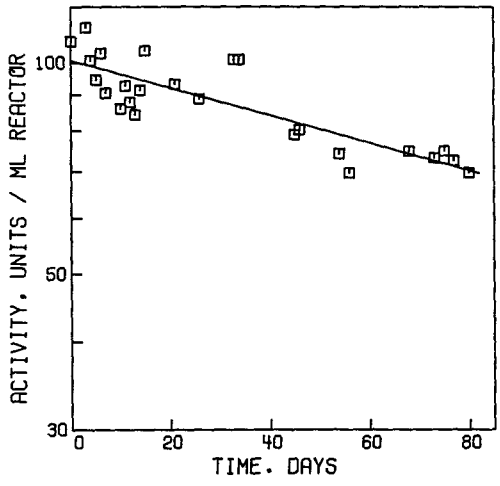

FIG. 6. Decay of activity at 40°C of glucoamylase immobilized on porous glass in a plug-flow reactor. Feed was 475 mg/ml maltose in 0.05 M acetate buffer at pH 4.25. Half-life given by the best straight line is 150 days. The "unit" of activity is 1 μmol product/min.

half-life 4 times ours. The most significant difference between the porous glass used is probably the pore size, being 55 nm in our case and 35 nm in theirs. These results suggest that 35 nm is better for glucoamylase.

DESIGN

To demonstrate the consequences of these deliberations, we have chosen to design a plant to produce ten million pounds per year of glucose (dry weight) from a 50% wt/wt solution of maltose. The quantity is realistic, but of course the substrate is not. The objective of our work is to draw broad generalizations from the data, rather than to pave the way to industrial utilization of this particular process. As Pitcher and Weetall politely put it (9), our estimates of cost are not "based on data comparable to potential commercial usage."

Parameters used in the design are shown in Table 2. The word "nominal" signifies that these values were used unless otherwise shown in the following tables. The cost of enzyme is based on "20¢/hundredweight" for a batch process to hydrolyze starch with glucoamylase, increased to account for the 65% retention of activity that we obtained on immobilization.

TABLE 2. **Nominal Input Parameters for the Design**

Cost of enzyme	$C_E = \$0.5$/million units
Cost of reagents	$C_R = \$100$/kg
Cost of carrier	$C_S = \$20$/liter
Cost of reactor shell	$C_D = \$2500$/m
Amount of reagents needed	$R = 0.1$ kg/liter
Operation time per year	$h = 330$ days
Fraction of carrier capacity remaining after regeneration	$L = 0.8$
Rate of depreciation	$Dp = 0.1$/year
Empty cross section of reactor	$A = 1500$ cm^2
Number of modules on-stream	$N = 10$

The cost of the carrier is based on the magnitude of current statements from suppliers on carriers intended for use with immobilized enzymes; our data, on which the design is based, were obtained with porous glass, which is not on the market for use with immobilized enzymes, but Corning Glass has said that their new controlled-pore ceramics have roughly the same properties. The cost of the reactor shell was obtained from Peters and Timmerhaus (15) (brought up to date by the Marshall and Stevens index) for a cross section of 1500 cm^2 (which gave a reasonable aspect ratio for the more important cases developed here).

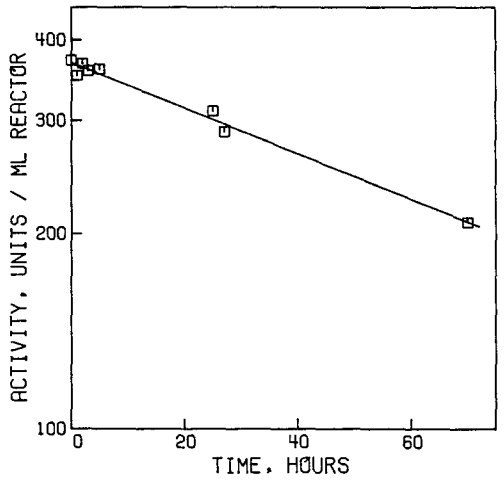

FIG. 7. Decay of activity at 60°C of glucoamylase immbolized on porous glass in a plug-flow reactor. Feed was the same as in Fig. 6. Half-life given by the best straight line is 85 h.

The amounts of reagents needed are based on the recipe in the literature (8) and used many times. The costs of silane and glutaraldehyde were used. Our own experience with sand (16) suggests that several orders of magnitude less may be possible, and this will have an important effect on the economic results. The problem of loss of carrier capacity on regeneration was first stated for us by Howard Weetall of Corning Glass; the value of 20% loss at each regeneration is our own nominal choice.

The results for the "recovered carrier" case are shown in Table 3. Two parameters were varied in the calculations: carrier cost and temperature. In the first two columns, the effect of carrier cost was probed, the "nominal" $20/liter against an estimated $300/liter for porous glass. The results are decisive. it is clear that the cost of carrier is a very important variable.

The first and third columns show the influence of temperature. It is clear that temperature is a very important variable, and 40°C is clearly superior. The only problem is that the cycle time is 2 years, which is a rather long time, considering the frequency of fluctuations in market demand. Following up this lead, we calculated the optimum temperature; results are shown in the fourth column. The optimum temperature is 23°C, and the total cost is $\frac{1}{2}$ that at 40°C, 1/20 that at 60. Again, cycle time is a problem, reaching 15 years in this case, a rather unrealistic value. Thus, it seems that low temperatures are best, but they introduce awkward problems in cycle time.

There are other problems with low temperature, too, such as rapidly increasing viscosity of high-concentration streams. Further, maximum microbial growth will occur near 40°C, which is an argument in favor of keeping the temperature above that point. Finally, the very long cycle times

TABLE 3. **Design Using the "Recovered Carrier"**

Temperature, °C	60	60	40	22.6 (optimum)
Cost of carrier, $/liter	20	300	20	20
Cycle time				
number of half-lives	9.0	11	3.9	1.1
days	32	40	600	4,800
Annual costs, $				
enzyme	1,700	1,700	150	34
reagents	93,000	93,000	8,400	1,800
carrier	37,000	560,000	3,400	740
reactor shell	1,600	2,100	2,800	5,000
TOTAL	130,000	650,000	15,000	7,600
Enzyme cost for batch process, $	20,000			

associated with low temperature will make deactivation by other than thermal means more conspicuous, means such as poisoning by trace quantities of heavy metals, and this has not been taken into account in the development here. Thus, it may be sensible to pick a temperature below which one is not willing to operate on the basis of these other considerations, and find the optimum values of the other parameters at this temperature.

The results for the "dissipated carrier" case are shown in Table 4. Three parameters were varied in the calculations: cost of the enzyme, cost of the reagents, and temperature. The first column should be compared with the first column in Table 3, all parameters being the same: the conclusion is that the costs are not much different, and so the major contribution of the more complex theory is in the more refined estimates of the operating parameters, rather than in the more refined estimates of cost.

The effect of enzyme cost is seen by comparing the first and second columns. It is clear that the cost of enzyme is incidental, the other factors being what they are. The effect of reagent costs is seen by comparing the first and third columns. It is clear that the cost of reagents is very important in this case, so that potential reduction of the amounts of reagents could be important.

To search out a lower limit to temperature that still might be modestly realistic, we set an upper limit to cycle times of 100 days and calculated the temperature at which this cycle time is optimum. The result is shown in the fifth column, a temperature of 49°C, which is not unreasonable. The total

TABLE 4. **Design Using the "Dissipated Carrier"**

Temperature, °C	60	60	60	49
Cost of enzyme, $/million units	0.5	10	0.5	0.5
Cost of reagents, $/liter	10	10	1	10
Cycle time				
number of half-lives	7.3	7.6	5.9	5.5
days	26	27	21	100
Number of cycles per module charge	16	16	17	11
Annual costs, $				
enzyme	1,700	34,000	1,700	340
reagents	93,000	93,000	9,400	37,000
carrier	38,000	38,000	39,000	16,000
reactor shell	4,400	4,600	3,800	5,000
TOTAL	140,000	170,000	54,000	58,000
Enzyme cost for batch process, $	20,000	400,000		

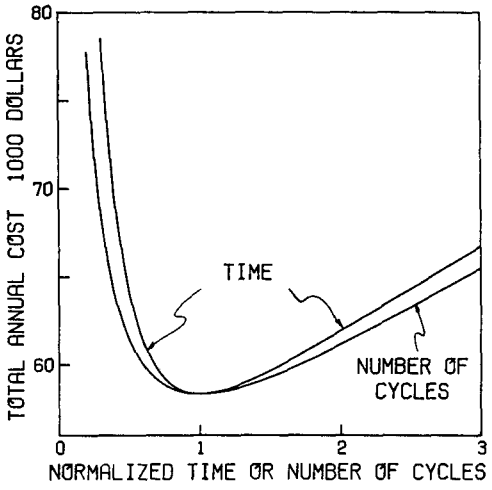

FIG. 8. Sensitivity of total cost to deviations from optima. Abscissa is cycle time or number of cycles divided by its optimum value.

cost, while several times that at 40°C, is a fraction of that at 60, so that this possibility seems well worth pursuing.

Let us take the results of the calculation at 49°C as being the most practical, and observe the consequences. First, it should be noted that the reagents are the largest contributor to cost; second, the enzyme is the least. If the enzyme were 20 times as expensive, as in the second column, the situation would not be much changed. Improvements in reagent economics would leave the carrier as the largest cost, and this factor is not likely to disappear in the near future.

The sensitivity of the total cost to variations from optimum of cycle time and number of cycles per carrier module is shown in Fig. 8. Each curve is based on the optimum value of the other variable. Cost is not overly sensitive to either variable; each can be changed by almost a factor of 2 either way without causing more than 5% increase in the total cost.

CONCLUSIONS

From the work, we conclude the following:

1. Cost is highly sensitive to temperature. Optimum temperature is low, close to room temperature, but cycle times are absurdly long. The best

policy may be to settle on a maximum reasonable cycle time, such as 100 days, and operate at the temperature that gives this optimum time.

2. The largest contributions to cost are reagents and carrier, even for an expensive enzyme.

3. Total cost is not very sensitive to large deviations of cycle time and number of cycles from optimum values.

4. The more complex "dissipated carrier" case gives more realistic values of operating parameters, but gives very little improvement in total cost estimation over the simpler "recovered carrier" case.

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