Pore Diffusion in Packed-Bed Reactors Containing Immobilized Glucoamylase

Conversion was measured in small packed-bed reactors containing immobilized glucoamylase in a range of variables in which diffusion was an important limitation. Three sets of data involving two carriers and two temperatures agreed satisfactorily with the theory of simultaneous reaction and diffusion.

Several reactors were designed for 99% conversion, and the results show that diffusion is a small problem when feed concentration is high but a serious problem at low concentration, requiring careful design of the catalyst. The particle size needed to eliminate the effect of diffusion is shown as a function of length.

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SCOPE

Enzymes are now being immobilized in porous carriers in commercial operations. If the carrier particle is too large or the activity too great, diffusion of reactants or products limits the rate, and the full capacity of the enzyme is not realized. Industrial planners must design enzyme carrier complexes which balance this problem against other costs.

The theory of combined reaction and diffusion has been well developed by persons in the petroleum industry and by academic people responsive to their problems, starting with the poineering work of Thiele (1939) and the later contributions of Aris (1957, 1969), Roberts and Satterfield (1965), Satterfield (1970), Petersen (1965), and others. An interesting history of the field was given by Thiele (1967), and the broader implications of the work were put in striking perspective by Weisz (1973). Application to biochemical reactors has been made by Kobayashi and Moo-Young (1973), whose first-order reaction simplifies the analysis; by Marsh et al. (1973), who ground porous glass to eliminate the influence of diffusion; by Kasche and Bergwall (1974); and by Toda and Shoda (1975), who studied trapped microbial cells.

The object of the work reported here was to find how serious diffusional resistance is in particular enzyme carrier complexes and to apply the theory to find the consequences of diffusion in the design of packed-bed reactors containing immobilized enzymes. To this end, effectiveness factors for glucoamylase immobilized on two agarose carriers were measured, and several reactors utilizing these catalysts were designed to show the impact of diffusion.

CONCLUSIONS AND SIGNIFICANCE

Data on the hydrolysis of maltose catalyzed by glucoamylase immobilized on two different carriers at two different temperatures agree reasonably well with the classical theory of simultaneous reaction and diffusion, corroborating for the case of immobilized enzyme the result of Toda and Shoda for trapped microbial cells.

For high concentration feed streams such as those found in chemical and food processing, enzyme carrier complexes having the fairly high activities observed here suffer only a small loss of effectiveness because of diffusion. However, lower concentration or higher activity applications may present serious diffusion problems, which increase costs.

Effectiveness factor can be maintained near unity in any design by judicious selection of activity level and particle diameter. This may call for a reactor with more than one catalyst, a high activity catalyst in the feed sections, and a less expensive low activity catalyst or smaller particle size in the exit sections.

Enzymes generally approach kinetics first order in reactant concentration as concentration is reduced, which means that the effectiveness factor of an immobilized enzyme catalyst will approach a lower limit as concentration decreases through a reactor. Thus, one may design a catalyst for a low concentration application, such as treatment of wastewater, which will be free of diffusional resistance for all concentrations.

MATERIALS AND METHODS

Glucoamylase from *Aspergillus niger* was obtained from Novo Enzyme Corporation and fractionated by gel permeation chromatography on 10% agarose in order to remove a small, high molecular weight impurity and a large, low molecular weight fraction.

Substrate used in all the tests was maltose, buffered by acetate buffer, pH 4.5. Glucose produced by the reaction

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was determined in the high concentration range by glucose oxidase in a Beckman glucose analyzer. In the middle concentration range, samples were incubated for 1 hr at 40°C with glucose oxidase, peroxidase, and *o*-dianisidine, conditions sufficient to oxidize almost all the glucose and produce an optimal absorbance with a maximum at 430 nm. The intensity of the absorbance was increased and the maximum shifted to 528 nm by then adding concentrated sulfuric acid. In the lowest concentration range, a mixture containing ten times the usual concentrations of these enzymes was incubated with the sample at room temperature in the cuvette of the spectrophotometer, and the rate of development of absorbance at 430 nm was determined. Activity is reported in units, defined as the number of micromoles of substrate reacted per minute.

Agarose is a linear polysaccharide obtained by separation from agar and containing very few charged groups, a property which has made it useful in chromatography. Percentage designations indicate the approximate concentration of agarose in the spherical beads. Thus, 4% agarose beads are approximately 96% void volume, which is useful in a study of diffusion. The beads maintain their shape by hydrogen bonds between contacting strands. Effective pore size, while not known, must be very large, as there appears to be little restriction of enzyme movement through the beads. The 4% beads used here were obtained from Pharmacia Fine Chemicals and the 10% beads from Bio-Rad Laboratories.

Before covalently binding the enzyme, the agarose was cross linked with epichlorohydrin (Porath et al., 1971), a step which we found necessary to obtain satisfactory stability of the immobilized enzyme at 50°C. Each material was then fractionated by elutriation in a conical vessel to give more uniform particle sizes suitable for a comparison of data to the theory. Particle size distributions were determined with a microscope equipped with a graduated reticule, and in each case the standard deviation of the distribution was about 8% of the mean, showing a reasonably narrow range of sizes. Mean diameter was 63 μm for the 4% beads and 254 μm for the 10% beads. Finally, the enzyme was immobilized on the agarose by the cyanogen bromide technique (Axen et al., 1967).

The diffusion coefficient D of maltose in 10% agarose at 50°C was measured by forming a slab of agarose about 2 mm thick, equilibrating the slab with a high concentration of maltose, then immersing it in a stirred vessel of water and measuring maltose concentration in the vessel as a function of time. The slab was formed by removing excess water from beads on a sintered glass filter, heating the beads to about 90°C, spreading the viscous fluid formed on a hot glass plate with a hot glass rod, and allowing it to cool. There was no change in concentration of agarose during this operation, as shown by the fact that a second phase did not appear.

The slope of the straight line on a semilog plot yielded a value of D of 3.1×10^{-4} cm²/min at 50°C and a tortuosity factor not significantly different from unity. The value of D at other temperatures was estimated by assuming that the group $D\mu/T$ is constant.

Effectiveness factors were determined in packed-bed reactors. When diffusional resistance is important in the interior of a porous catalyst particle in this size range, it almost always is important in the interstices of the bed also, and this part of the resistance must be eliminated for comparison with the classical theory. In experiments to this end, conversion in a packed-bed reactor was determined as a function of flow rate, and that flow rate sufficient to reach the limiting upper conversion was chosen for the experiments in which effectiveness factor was determined.

Extent of reaction was limited to 10% by regulating the size of the bed, which allowed the differential reactor equation to be used:

$$v_i = F(C_1 - C_2)/V \tag{1}$$

Following the usual practice, there is no void fraction ϵ in Equation (1), which means the basis is the total reactor volume, not the spherical volume of the beads alone. In comparisons with the diffusion theory, reaction velocities from Equation (1) must be divided by $(1 - \epsilon)$.

Effectiveness factor is defined as the ratio of reaction velocity to that in the absence of diffusional resistance v^*:

$$\eta = v_i/v^* \tag{2}$$

Thus, it is necessary to know v^*, and this raises some problems as there is no consensus in the literature on the impact of immobilization on the kinetic behavior of an enzyme. Papers are available showing changes of kinetic constants in both directions and some showing no changes, but unfortunately many of these results are clouded by the uncertain extent of diffusional influence.

Enzyme kinetics can be divided for discussion into two parts, the limiting reaction rate at high concentration V_{max} and the function multiplying V_{max} which describes the influence of reactant concentration $f(c)$:

$$v^* = V_{max}\, f(c) \tag{3}$$

As a working hypothesis, we assume that $f(c)$ is the same for the immobilized and dissolved enzymes. In unpublished experiments in this laboratory involving glucoamylase bound to nonporous glass and Sephadex nonporous to the enzyme, this was observed. Essentially the same result has been reported by Ramachandran and Perlmutter (1976) for glucose oxidase and urease bound to nonporous glass. Differences between the values of V_{max} for the dissolved and immobilized enzyme may be interpreted as showing unfavorable orientations of the bound enzyme molecule; with several enzymes and binding methods, we have observed a recovery of activity following immobilization of around 50%, which is compatible with the random orientation argument, and values on this order were observed in the tests reported here. Using this hypothesis, then, the reaction rate of the immobilized enzyme in the absence of diffusion v^* was obtained by multiplying the value of V_{max} for the immobilized enzyme by $f(c)$ of the dissolved enzyme.

Theoretical values of effectiveness factor were calculated by numerical solution of the equation

$$\frac{1}{r^2}\frac{d}{dr}\left(r^2 D \frac{dc}{dr}\right) = v^* \tag{4}$$

and application of the results to the equation

$$\eta = \frac{3D}{R\, v^*}\left(\frac{dc}{dr}\right)_R \tag{5}$$

Effectiveness factors are here plotted against the modulus devised by Bischoff (1965) and independently by Aris (1965)

$$m = \frac{R}{3\sqrt{2}}\,\frac{v^*}{\left[\int_0^c Dv^*\, dc\right]^{1/2}} \tag{6}$$

because it collapses lines for all kinetics to the same high modulus asymptote, which greatly simplifies the comparison of theory and experiment.

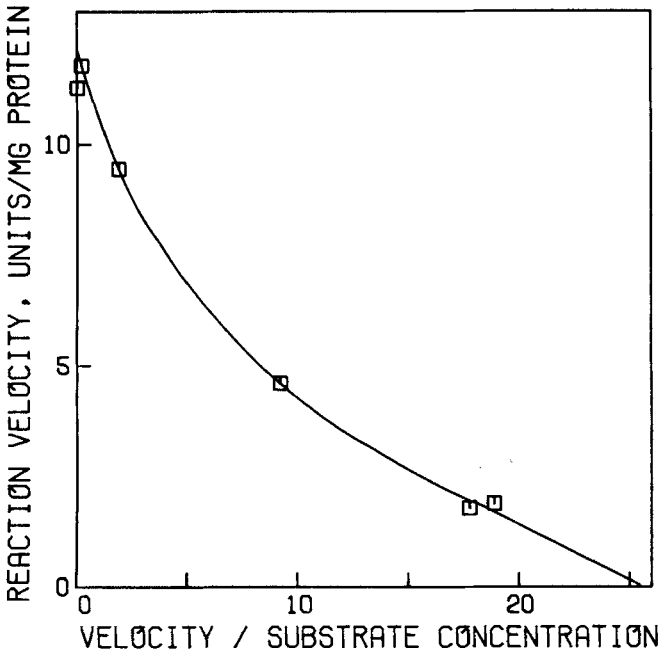

Fig. 1. Hofstee plot for dissolved glucoamylase, from initial velocity measurements. Substrate is maltose in 0.05 M acetate buffer at pH 4.5, 40°C. Dimensions of abscissa are (units) (milliliters)/(milligram protein) (milligram maltose).

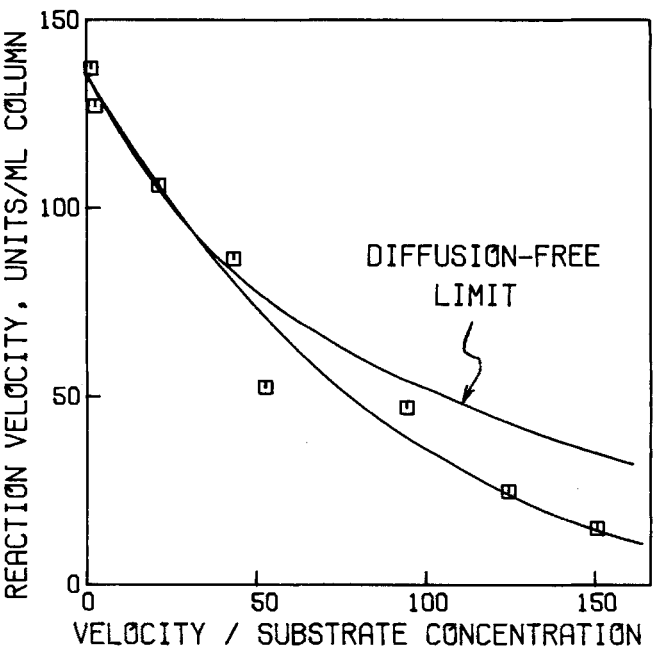

Fig. 2. Hofstee plot for glucoamylase immobilized on 4% agarose. Data obtained in a plug-flow reactor fed maltose in 0.01 M acetate buffer at pH 4.5, 40°C.

EXPERIMENTAL RESULTS

Results pertaining to the dissolved enzyme, the immobilized enzyme, and finally the effectiveness factor are given in this section.

The Dissolved Enzyme

Figure 1 gives the initial velocity data in a batch reactor for the dissolved enzyme at 40°C on a Hofstee plot. The uppermost point is an average of about ten separate determinations; the deviation of the adjacent point is representative of the scatter of the data. Hofstee plots (Hofstee, 1952) are convenient for this work because concentrations from zero to infinity are encompassed within the limits of the axes, because diffusional effects shift the lines more than on other plots (noted by Engasser and Horvath, 1973), and because points spaced well for a study of diffusion are also conveniently spaced on the Hofstee plot.

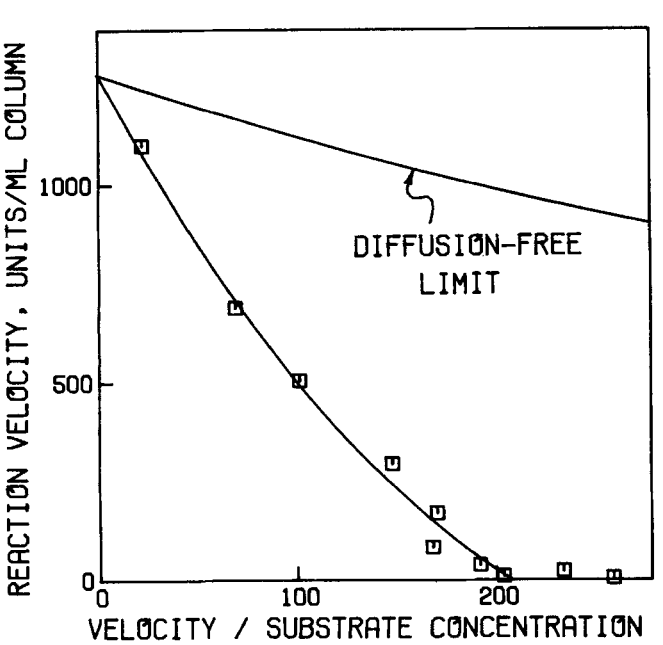

Fig. 3. Hofstee plot for glucoamylase immobilized on 10% agarose. Data obtained in a plug-flow reactor fed maltose in 0.01 M acetate buffer at pH 4.5, 50°C.

Characteristics of Immobilized Glucoamylase

Figure 2 presents data for glucoamylase immobilized on 4% agarose in a packed-bed reactor. The line giving the diffusion free limit is the product of V_{max} for the immobilized enzyme, obtained by extrapolating the best line through the data of Figure 2 to the ordinate, and $f(c)$ for the dissolved enzyme, which was obtained from the data in Figure 1. Since the slope of a line from the origin is substrate concentration, the value of effectiveness factor is the ratio of distances from the origin to the two lines at that concentration. It may be seen that there is small impact of diffusion on the reaction rate for the case shown in Figure 2.

Figure 3 gives results for glucoamylase immobilized on 10% agarose. The data deviate much more from the diffusion free limit for two reasons: namely, the particles have a diameter four times that of the 4% agarose preparation, and the measured maximum activity of the catalyst was four times as great per unit volume of reactor, in part because there is about 2.5 times as much mass of carrier per unit volume to which the enzyme can bind, and in part because the dissolved enzyme preparation used in the immobilization had been further purified.

Activity was measured as a function of temperature for the dissolved enzyme and the enzyme immobilized on 10% agarose. The reactant concentration used with the immobilized enzyme was the highest one used in the data shown in Figure 3, which minimized diffusional resistance. Data for both preparations were well fit by the Arrhenius equation, yielding activation energies of 15.5 and 14.2 kcal/mole, respectively. The closeness of these figures further corroborates the relative freedom of the immobilized enzyme from diffusional resistance at this high reactant concentration.

TABLE 1. IMPACT OF DIFFUSION ON RESIDENCE TIME

Agarose conc., %	T, °C	Feed concentration, mg/ml	Residence time	Ratio of actual time to diffusion free limit
10	50	500	2.2 min	1.014
10	50	1	5.0 min	3.9
10	50	0.01 and below	4.2 s	7.4
4	40	0.01 and below	6.4 s	1.19

Effectiveness Factor

Effectiveness factor η as a function of the Bischoff-Aris modulus is shown in Figure 4. As noted above, this modulus brings the curves for different kinetics very close together. In the absence of inhibition and activation effects, the influence of reactant concentration on most enzymic reactions can be approximated by an equation first order in concentration at low concentrations and zero order at high concentrations. These approximations apply as well to this enzyme. The result is that curves for the theoretical prediction of effectiveness factor for the three cases shown in Figure 4 all fall between the curves for zero order and first order, a difference which is no larger than the experimental error.

An interesting facet of first-order reactions is that the theoretical effectiveness factor is independent of concentration (Roberts and Satterfield, 1965). This means that in a reactor containing immobilized enzyme, the effectiveness factor in the region near the exit, where the concentration of reactant will be low and the kinetics effectively first order, will approach a limiting lower value characteristic of the enzyme carrier complex. Thus the limiting upper values of modulus for the three sets of data in Figure 4 are 0.61, 5.2, and 6.8, corresponding to limiting lower values of effectiveness factor of 0.84, 0.17, and 0.13. The implication is that it is possible to design an enzyme carrier complex that will not go below an arbitrarily set value of effectiveness factor by regulating the activity and particle size of the catalyst, the reason being that the kinetics always approaches first order as concentration becomes low.

The data of Figure 4 are in reasonable agreement with the theory, and to the extent that they are, they constitute a check of the internal consistency of the data and the usefulness of the assumption that the effect of concentration is the same for the dissolved and immobilized enzymes. The values of effectiveness factor are sensitive to the precision of the measurement of the lowest concentrations, and it appears that the precision of these concentrations does not equal that of the others.

DESIGN PROBLEM

To demonstrate the consequences of the measurements, a plant is here designed to accomplish the same reaction. A conversion of 99% was set, and several feed concentrations and two different catalysts were studied.

The reactor equation for the plug-flow reactor is

$$-F\,dc = v_i\,dV = \eta\,v^*\,dV \qquad (7)$$

The superficial residence time, or space time t is

$$t = \frac{V}{F} = \int_{c_2}^{c_1} \frac{d(c)}{v_i} \qquad (8)$$

This can be broken into two terms, the first of which gives the diffusion free limit and the second of which gives the extra residence time caused by diffusion:

$$t = \int_{c_2}^{c_1} \frac{dc}{v^*} + \int_{c_2}^{c_1} \left(\frac{1}{\eta} - 1\right) \frac{dc}{v^*} \qquad (9)$$

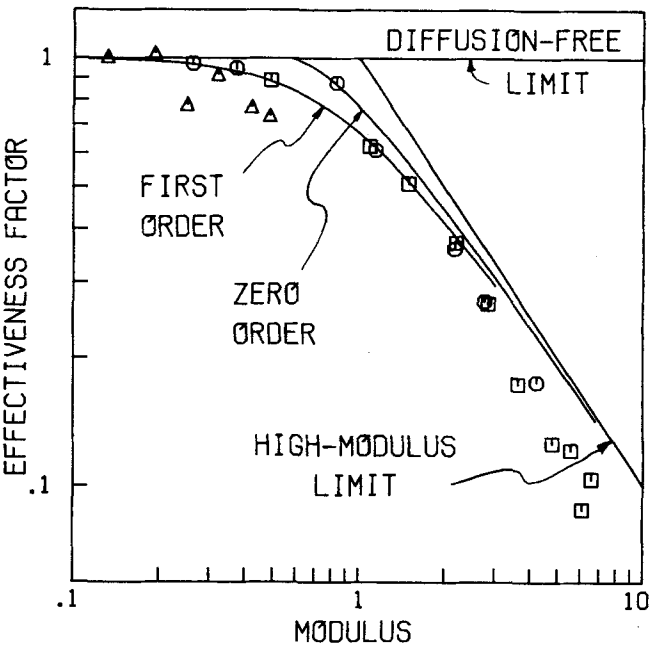

Fig. 4. Effectiveness factor as a function of modulus, glucoamylase immobilized on agarose. (△), 4% agarose, 40°C, R = 31 μm; (○), 10% agarose, 40°C, R = 127 μm; (□), 10% agarose, 50°C, R = 127 μm.

Three feed concentrations were chosen, shown in Table 1, the highest one to represent feed streams found in the processing of foods or chemicals, and the others to represent the dilute streams characteristic of waste treatment.

At the highest concentrations, even the most active carrier, 10% agarose at 50°C, suffered only a 1% increase in residence time because of diffusion. Thus, catalysts having the high activity observed here are not likely

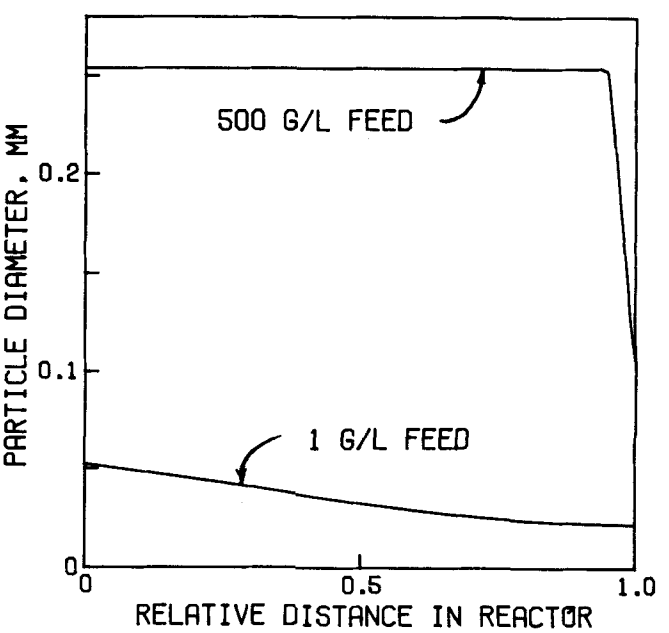

Fig. 5. Diameter of particle needed to keep effectiveness factor near unity, design case involving 10% agarose at 50°C.

to cause problems in high concentration processing. However, at low concentrations the consequences are severe. The 10% agarose at 50°C has about reached its first-order limit at a maltose concentration of 0.01 mg/ml, which means that the effectiveness factor is constant for all concentrations below this. The actual residence time is 7.4 times the ideal, no matter how low the concentration, and the residence time is constant at 4.2 s for the 99% conversion specified.

By comparison, the 4% agarose at 40°C is about one tenth as active, and even at the low concentration extreme, it causes only 19% increase in residence time. This could be reduced further with a smaller particle or lower activity loadings. Thus, one may control the parameters of the catalyst to limit the effect of diffusion to any arbitrary value.

Given the waste of enzyme by the 10% agarose catalyst at low reactant concentrations, the question may be asked, what can be done to improve performance? Two primary variables determine the relative importance of diffusion in a catalyst, the particle size and the activity of unit amount of carrier. As an example, the particle size needed to maintain an effectiveness factor near unity was calculated as a function of distance through the reactor. Diameter was set at 254 μm maximum, the same as in the experiments, until the modulus reached unity, after which diameter was reduced continuously to maintain the modulus at unity.

The result for the first two cases in Table 1 is shown in Figure 5. Little change is needed for the high concentration feed, which is in agreement with the small increase in reactor volume needed if the diameter were constant. For the low concentration feed, however, very small particle diameters are needed, starting at 50 μm and decreasing to about 20 μm at the exit. These sizes are probably too small to be practical, indicating that lower activity would be better for such an application, and thus a less expensive carrier with lower capacity for enzyme could be used. In practice, one would plan not for continuously changing particle size, but for a reactor with more than one section, with high-activity catalyst in the entrance sections and low activity or small particle size in the exit sections.

CONCLUSIONS

From the work, we conclude the following:

1. Reaction rate data for the hydrolysis of maltose by glucoamylase immobilized on agarose are reasonably well fit by the classical theory of simultaneous reaction and diffusion.
2. Because enzymes approach first-order kinetics as concentration is reduced, it is possible to design enzyme carrier complexes that have an arbitrarily chosen minimum effectiveness factor.
3. For high concentration feed streams, diffusion will be little problem, even with active carriers. For low concentration feeds, however, enzyme carrier complexes will have to be carefully designed.
4. Through judicious choice of activity level and particle diameter, effectiveness factor of an enzyme carrier complex can be maintained near unity. For low concentration feeds, this will allow inexpensive carriers of low binding capacity to be used.

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NOTATION

c = concentration of reactant, mg/ml
c_1, c_2 = reactant concentration at entrance and exit of the reactor, mg/ml
D = effective diffusion coefficient in particle, cm^2/min
F = feed rate to reactor, ml/min
m = modulus, dimensionless, defined by Equation (5)
r = radius parameter
R = radius of spherical bead, cm
t = superficial residence time, or space time, min
T = temperature, °K
v_d, v_i = reaction velocity, mg maltose/(min)(ml), for dissolved and immobilized enzyme, respectively
v^* = diffusion free reaction velocity, mg maltose/(min)(ml)
V_{max} = maximum reaction velocity, mg maltose/(min)(ml)
V = volume of reactor, ml
ϵ = void fraction in packed-bed reactor, dimensionless
η = effectiveness factor, dimensionless
μ = viscosity of fluid, any units

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