

Study on The Performance of a Tubular Enzyme Reactor

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ABSTRACT

The residence time distribution of fluid passing through a tubular reactor was studied to predict the performance of a tubular enzyme reactor. It was found that there was a great amount of dispersion from the ideal plug-flow over the range of flow rate of 15 ml/min to 78 ml/min. Sucrose hydrolysis by yeast invertase has been selected as an enzymatic model reaction in a tubular reactor. The kinetics of invertase were studied by the observation of the initial rates of reaction at various substrate concentrations.

A mathematical model which described the degree of conversion of a tubular enzyme reactor as a function of flow rate is developed. The expression of the rate of reaction is approximated by a linear function using Maclaurin theorem. The dispersion model shows good agreement with the observations on sucrose hydrolyzed by invertase in a tubular reactor.

INTRODUCTION

Interest in the application of enzyme reactors to synthesis various products by means of enzymatic reactions has been obviously increased (Bowski and Shah, 1972; Mansfeld and Schellenberger, 1987; Bickerstaff, 1987). An enzyme reactor is basically a container in which a reaction catalysed by free or immobilized enzyme take places. Many different reactors have been used with free or immobilized enzyme. The choice of reactors depend on several factors such as, reaction kinetics, operation requirement, catalyst replacement etc (Lily and Dunnill, 1971). Choice of the most appropriate type of reactor and their efficient design and operation is very important in supporting successful used of enzymes in industries.

In typical chemical engineering reaction theory, there are two classical types of ideal continuous reactor that describe most of the real reactor namely continuous stirred tank reactor (CSTR) and plug flow reactor (PFR). The reactor flow patterns range from the extreme type of perfectly mixed to the ideal plug

flow. If the flow of the fluid through a reactor can be described as one of those two ideal types, it is relatively simple to predict the performance of the enzyme reactor. However, real reactors actually never follow the ideal patterns. Non-ideal flow patterns are more realistic condition encountered in real large scale process reactor (Balley and Ollis, 1986). The deviation from these two ideal flow patterns may be considerable.

In the case of non-ideal flow, the elements of fluid move through the reactor at varying rates and require different length of time to pass through the reactor. The distribution of these times for the fluid leaving the reactor is called Residence Time Distribution (RTD) of fluids (Livenspiel, 1972). Residence Time Distribution of fluids gives an important information of the actual velocity profile of the fluid in the reactor, how far it is from those two ideal flow patterns. Variations of residence time lead to several important consequences, especially an increase in size of reactor for a given out put as deviation from plug-flow becomes significant (Kobayashi and Moo Young, 1971). Thus, by knowing precisely what is happening within the reactor, it might be possible to make the right correction of the simple model and obtain more accurate description.

Several mathematical models have been derived which describe deviation from ideal flow behavior. Models may be able to predict the behavior of reactors gives an information of the way in which improvement can be made. Therefore it would be useful for process design and control purpose. One model which is useful for equal application and also relatively simple mathematically is the Dispersion Model (Levenspiel, 1972). The parameter which measure the extent of dispersion is called Dispersion Number.

Sucrose hydrolysed by yeast invertase has been selected as an enzymatic model reaction in a tubular reactor. Invertase is one of the commercial important enzyme. The applications of invertase have been found in the food and confectionary industries (Priest, 1984). In the course of these works, the residence time distribution of fluid passing through a tubular reactor and

kinetics of invertase were studied. Using those information, a simple mathematical model for prediction of the performance of a tubular reactor, which described the degree of conversion as a function of flow rate was developed and then compared to the experimental observed.

THEORY

The kinetic expression for ideal plug-flow reactor and CSTR are described in equations 1 and 2 respectively.

$$tPF = f [S_0]/V_{max} - (K_m/V_{max}) \ln (1 - f) \quad (1)$$

$$tCSTR = f [S_0]/V_{max} + (K_m/V_{max}) f(1 - f) \quad (2)$$

In reality the flow of fluid varies from those two ideal cases. Chemical reaction with dispersion can be described by the equation below (Levenspiel, 1972):

$$(D/UL) d^2S/dZ^2 - dS/dZ - tr = 0 \quad (3)$$

In enzymatic reaction, the rate of reaction which is a non linear reaction rate, is:

$$r = V_{max} S/(K_m + S) \quad (4)$$

Thus it is difficult to get a numerical solution of equation 3. However, the expression could be approximated by a linear function using Maclaurin theorem around $S = S_1$, and S_1 is a point of linearizing which has been defined as 0.09. Thus the approximate rate of reaction is:

$$r = mS + b \quad (5)$$

Where $m = V_{max} K_m/(K_m + S_1)^2$, and $b = m S_1^2/K_m$

Thus by means Maclaurin approximation of the rate, the second order non homogeneous differential equation:

$$(D/UL) d^2S/dZ^2 - dS/dZ - t(mS + b) = 0 \quad (6)$$

Let, $(D/UL) = \alpha$; and $d/dZ = \lambda$, thus equation 6 becomes,

$$\alpha \lambda^2 S - \lambda S - tmS = bt \quad (7)$$

The characteristic equation is

$$\alpha \lambda^2 - \lambda - tm = 0 \quad (8)$$

λ_1 and λ_2 can be calculated using quadratic equation. Homogeneous solution of substrate concentration is

$$S_h = A e^{\lambda_1 z} + B e^{\lambda_2 z} \quad (9)$$

Let us assume that the particular solution S is constant, then

$$d^2S/dZ^2 = 0 = dS/dZ = 0; \text{ therefore } S = -b/m \quad (10)$$

The analytical solution of unconverted substrate concentration was obtained below. Integration contents A and B can be obtained from the boundary condition:

$$S = A e^{\lambda_1 z} + B e^{\lambda_2 z} - b/m \quad (11)$$

$$\text{When } Z = 0, S = S_0, \text{ thus } S_0 = A + B - b/m \quad (12)$$

$$\text{When } Z = L, dS/dZ = 0, \text{ thus}$$

$$0 = A \lambda_1 e^{\lambda_1 L} + B \lambda_2 e^{\lambda_2 L} \quad (13)$$

A and B can be calculated from equation 12 and 13.

Degree of conversion is defined as,

$$f = (S_0 - S) / S_0 \quad (14)$$

MATERIALS AND METHODS

Invertase (β -D-fructofuranosidase) from yeast (E.C.3.2.1.26) purchased from BDH Chemical Ltd was used as an enzyme model throughout these works. Kinetic investigations and enzymatic reaction in a tubular reactor were performed using pure-grade sucrose as a substrate from Sherman Chemical Ltd. Reagent for determination of glucose were purchased from Boehringer Mannheim GmbH Diagnostica.

Residence time distribution

The residence time distribution was determined by step-signal method of the stimulus response experiment (Levenspiel, 1972). Experiments were carried out at three different flow rates, 15 ml/min; 41 ml/min; and 78 ml/min. Initially, there was no tracer present in a steady flow of white fluid passing through C26/70 column from Pharmacia Fine Chemical. The column diameter and column length are 2.6 cm and 70 cm respectively. At time zero, the fluid was switched to the blue tracer and recorded the concentration of tracer in the exit stream by measuring its absorbance at a wavelength of 650 nm (PYE Unicam PU 8600 UV/VIS). The direct response is called F curve ($F = C/C_0$). C and C_0 are concentration of tracer and initial concentration of tracer respectively. The mean residence time for ideal fluid passing through a column at various flow rates was estimated ($t = \text{column volume/flow rate}$).

The dispersion number (D/UL) was determined by transforming F curve to C curve which obtained from the slope of F curve ($C = dF/dt$). The variance of a continuous distribution is

$$\sigma^2 = \sum t_i^2 C_i / \sum C_i - \{(\sum t_i C_i) / \sum C_i\}^2 \quad (15)$$

Dispersion number equals to $\sigma^2 / 2$, where $\sigma^2 = \sigma^2 / t$, and $\bar{t} = \sum t_i C_i / \sum C_i$

Enzyme assay

Kinetic studies were performed at $30 \pm 0.5^\circ\text{C}$ in sodium acetate buffer pH 4.8. Invertase (0.4% v/v) was added into the substrate solution containing sucrose concentration ranging from 0.03 M to 0.27 M and mixed quickly. The reaction was stopped by adding NaOH 2N into 1 ml working solutions, and then immersed them in $121\text{-}125^\circ\text{C}$ oil bath for 80 seconds. Glucose formed in the course of reactions was estimated by glucose oxidase peroxidase method. Sucrose concentration was determined with polarimetric technique (Egan *et al*, 1981).

Enzymatic reaction in a tubular reactor

The experiments were carried out in C 26/70 column with a jacket added purchased from Pharmacia Fine Chemical. Sucrose and enzyme solution from different reservoirs were pumped by peristaltic pump (Watson-Marlow 502S) with various flow rates varied from 30 ml/min to 71 ml/min, to the C16/70 pre-heating column before entering the reaction column. The reaction was carried out at $30 \pm 0.5^\circ\text{C}$, pH 4.8. The concentrations of sucrose and enzyme in the reaction column were 5% v/v and 0.4% v/v respectively. After steady state was established, the degree of conversion was evaluated at the end of the column by assaying the amount of glucose formed.

RESULTS AND DISCUSSION

Effect of flow rate on the residence time distribution

The result obtained for residence time distribution of three different flow rates namely 15.70 ml/min; 41.00 ml/min and 78.00 ml/min are summarized in Figure 1. It is clear that the velocity profiles of those three flow rates behave neither as plug-flow nor complete mixing system, but there are some dispersion from those two ideal cases. For ideal plug-flow pattern, fluid passing through the column at every point in uniform velocity. It means time needed (\bar{t}) by fluid to pass throughout the column is also the same. Thus, C/C_0 should be zero at time less than \bar{t} , and at time \bar{t} forward C/C_0 becomes 1. The dispersion number (D/UL) of those three flow rates are presented in Table 1. There is a tendency of increasing the dispersion number with the decrease in flow rate. Three

points may be not enough to see the relationship between dispersion number and flow rate. However, at least in the range of flow rate of 15 ml/min to 78 ml/min, the flow rate and dispersion number has a linear relationship. The dispersion number (D/UL) of Plug-flow and completely mixed flow are 0 and ∞ respectively (Livenspiel, 1972). Dispersion number of 0.2 represents a large amount of dispersion. It means that high degree of back mixing occurred during the flow. Thus the flow patterns are very far from the ideal plug-flow. Breaking the velocity profile with inert material may help to reduce the dispersion, and thus improve the reactor performance.

Considering the corresponding Reynold Number shown in Table 1, those three flow rates have very low Reynold Number, which are very much less than 2000. At Reynold Number values less than 2000, the flow regime is always laminar (Coulson and Richardson, 1977). Thus the distribution of velocity is not uniform, but varies at every point across a diameter of the column from zero at the wall to maximum at the center.

Table 1. Dispersion number and Reynold number as a function of flow rate

Flow rate (ml/min)	Residence Time (min) ^{a)}	Dispersion number (D/UL)	Reynold number ^{b)} (Re)
15.7	12.68	0.5081	12.81
41.0	9.07	0.4444	33.46
78.0	4.77	0.3264	63.65

a). Residence Time for ideal flow in C26/70 column

b). Reynold number, $Re = \frac{d u \rho}{\mu}$ (dimensionless)

Mathematical modelling

In enzymatic reaction, the rate of reaction, $v = r = \frac{V_{\max} S}{K_m + S}$, which is a nonlinear reaction rate. This expression can be approximated by linear function using Maclaurin theorem, around $S = S_1$. Thus the approximate rate of reaction is $r = mS + b$ (equation 5), where $m = \frac{V_{\max} K_m}{(K_m + S_1)^2}$, and $b = \frac{m S_1^2}{K_m}$. S_1 is a point of linearity which has been defined as 0.09 (i.e., a middle point in the substrate range during the reaction). From the kinetic studies of invertase catalysis hydrolysis of sucrose, K_m and V_{\max} values obtained which determined by Eadie-Hofstee plot are 0.05 M and 0.024 M/min respectively with the correlation coefficient of -0.9734. The values of m and b are calculated to be 0.0638 min^{-1} and 0.0103 M/min. Using K_m and V_{\max} values

observed, the actual rate of reaction (r) and the approximate reaction rate (r^*) at various substrate concentrations can be calculated from equation 4 and 5. The results are presented in Figure 2. It can be seen that approximate reaction rate adequately fits the actual rate of enzymatic reaction in the range of substrate concentration 0.07 M to 0.11 M.

Experimental validation of the mathematical model

The mathematical model was experimentally validated. Enzymatic reactions were carried out in a tubular reactor with various flow rates, namely 30 ml/min; 37.80 ml/min; 48.50 ml/min and 71.65 ml/min. The degree of conversion as a function of flow rate was calculated when steady state was established. The degree of conversion of ideal plug flow for corresponding flow rate has been calculated from equation 1.

Table 2 shows that the degree of conversion tends to decrease with the increase in flow rate. This reflects the corresponding increase in the residence time of fluid in the reactor. For a given reactor, the higher the residence time the longer the reaction takes place, thus the higher the degree of conversion. The residence time is a function of flow rate. High flow rate lead to low residence time therefore low degree of conversion and conversely.

As shown in Table 2, the experimental degree of conversion observed are very far from the one obtained by ideal plug flow. This shows the effect of dispersion. All flow rates used in this study have dispersion number more than 0.2. It means they have very great amount of dispersion from the ideal plug-flow. High dispersion causes low degree of conversion. The occurrence of back mixing will have significant effect, particularly when the reactions are operated at low S_0/K_m . It will drastically reduce the reactor performance. Thus larger reactor size should be used in order to get the same degree of conversion.

Table 2. Degree of conversion for enzymatic reaction in a tubular reactor

Flow rate (ml/min)	Residence Time (min)	Dispersion number	degree of conversion (a)	(b)
30.00	12.40	0.470	0.68	1.00
37.80	10.15	0.447	0.60	1.00
48.50	7.70	0.416	0.50	0.79
71.65	5.19	0.380	0.43	0.59

a). experimental results

b). ideal plug-flow

Comparing the predicted degree of conversion to the experimental observed as shown in Figure 3, the experimental results fit well the model. Despite the fact that the approximate reaction rate, r^* , deviates from the actual rate especially in higher degree of conversion as shown in figure 2, the prediction are very good.

Apart from linearization errors, however, this study shows that the residence time distribution experiment is verified giving very important information in the prediction of a tubular enzyme reactor. With those errors and the limited condition in this study, we can say that the dispersion model can be used to predict the performance of tubular enzyme reactor.

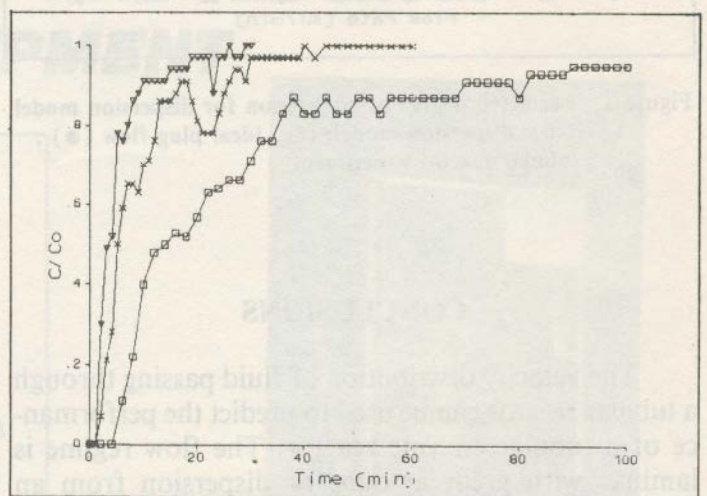


Figure 1. F curves of tracer with various flow rates (\square) 15.7 ml/min; (\times) 41.0 ml/min; (∇) 78.0 ml/min.

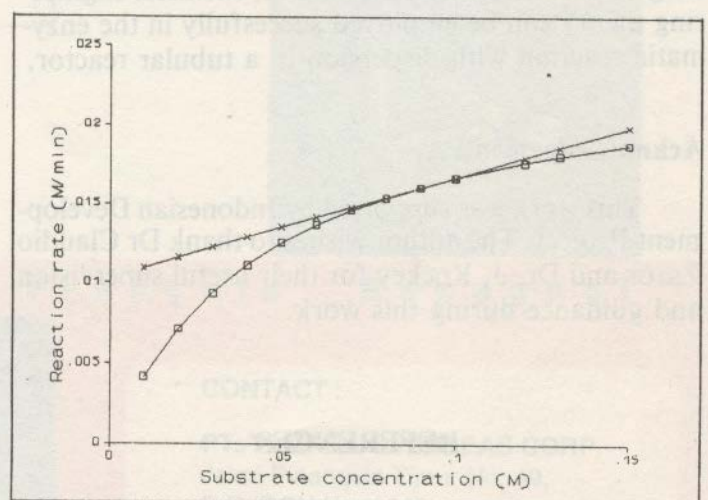


Figure 2. The actual rate of enzymatic reaction (r) and linear approximate reaction rate (r^*) in various substrate concentration (\square) actual rate of reaction; (\times) linear approximate reaction rate.

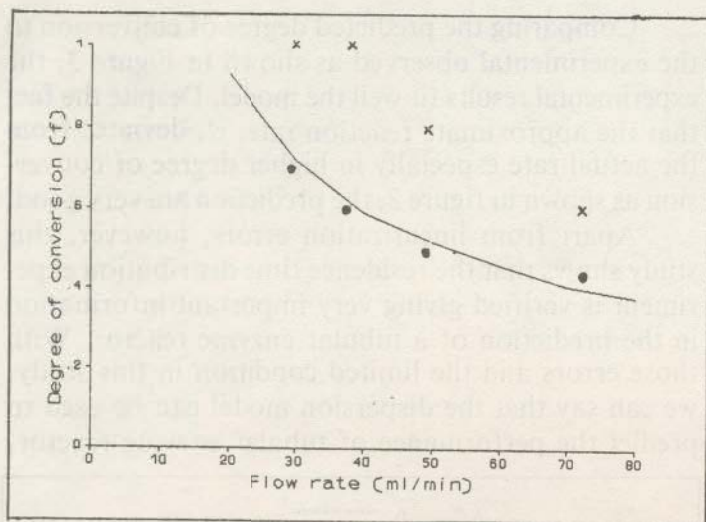


Figure 3. Predicted degree of conversion for dispersion model (-): dispersion model; (x) : ideal plug-flow (●) : tubular reactor experiment

CONCLUSIONS

The velocity distribution of fluid passing through a tubular reactor can be used to predict the performance of a tubular enzyme reactor. The flow regime is laminar with great amount of dispersion from an ideal plug flow. The dispersion model including a linear approximation of the reaction rates gives adequate prediction of substrate conversion within the range of flow rates observed. Thus, chemical engineering theory can be employed successfully in the enzymatic reaction with dispersion in a tubular reactor.

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ABBREVIATIONS

- tPF = mean residence time for plug flow reactor
 tCSTR = mean residence time for continuous stirred reactor
 f = degree of substrate conversion, $(S_0 - S)/S_0$
 K_m = Michaelis constant
 V_{max} = maximum initial rate of reaction
 S_0 = initial substrate concentration
 (D/UL) = Dispersion number (dimensionless)
 S = substrate concentration
 Z = $1/L$ (dimensionless)
 \bar{t} = mean residence time
 r = reaction rate
 r^* = approximate reaction rate
 S_h = substrate concentration in homogenous solution
 L = length of reactor column
 d = column diameter (2.6 cm)
 u = average velocity ($u = Q/A$)
 Q = volumetric flow rate
 A = area of flow ($\pi d^2/4$)
 ρ = density of liquid (2 kg/l)
 μ = viscosity of liquid (2×10^{-5} kg/cm/sec)