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Prediction of Continuous Reactors Performance Based on Batch Reactor Deactivation Kinetics Data of Immobilized Lipase

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Abstract Experiments on deactivation kinetics of immobilized lipase enzyme from *Candida cylindracea* were performed in stirred batch reactor using rice bran oil as the substrate and temperature as the deactivation parameter. The data were fitted in first order deactivation model. The effect of temperature on deactivation rate was represented by Arrhenius equation. Theoretical equations were developed based on pseudo-steady state approximation and Michaelis –Menten rate expression to predict the time course of conversion due to enzyme deactivation and apparent half-life of the immobilized enzyme activity in PFR and CSTR under constant feed rate policy for no diffusion limitation and diffusion limitation of first order. Stability of enzyme in these continuous reactors was predicted and factors affecting the stability were analyzed.

Keywords: batch and continuous reactors, deactivation kinetics, apparent half-life, diffusion control and enzyme stability

INTRODUCTION

The use of lipase enzyme for bioconversion of fats and oils to useful derivatives is of considerable importance in biotechnology [1]. One of the major drawbacks of the enzyme is that they loose their catalytic activity during the course of reaction, which is known as decay, inactivation or deactivation. Enzyme inactivation plays a significant role in biotechnological processes as rapid inactivation may adversely affect the efficiency of the bioprocess concerned [2,3]. The durability of the biocatalyst during continuous operation is called operation stability and is measured by its half-life *i.e* the elapsed time at which the catalyst activity is reduced to half of its original one. Half-life is an important parameter, that decides the economical feasibility of the bioprocess concerned [4]. Hence, there is a need for deactivation studies to predict the behavior of the continuous process under disparate conditions.

In the present work deactivation kinetics experiments were performed in a batch reactor at different temperatures with rice bran oil as substrate and immobilized lipase enzyme as biocatalyst. The experimental data were fitted in model equations, which were used for predicting continuous reactors performance under constant feed rate policy [5].

Influence of substrate concentration, initial conversion levels and mass transfer effects on apparent half-lives, predicted by model equations were discussed. Apparent half-life in constant feed rate policy was defined as the elapsed time at which conversion becomes half of the initial one. The predicted apparent half-lives were compared with that of the actual half-lives reported by various authors [6,7].

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MATERIALS AND METHODS

Materials

Lipase enzyme from *Candida cylindracea* (285 U/mg) was obtained from Sigma glass beads (2 mm, spherical, acid washed, Sigma) were used as enzyme support material for immobilization. All chemicals used in this work were reagent grade and were product of Nice chemicals, Cochin, India. Rice bran oil (Iodine value=90 FFA=0.3% (w/w)) was obtained from Sri Jayasakthi Rice & Oil Mills, Salem, India.

Immobilization of Lipase Enzyme

Lipase enzyme from *Candida cylindracea* was immobilized on 2 mm acid washed activated glass beads based on the method developed by Wu and Weng [8].

Lipase Activity

The activity of lipase is described in terms of lipase units (U). One unit (U) of lipase is defined as the amount of enzyme required to produce one micromole of free fatty acid in one minute under assay conditions.

Free fatty acids liberated were measured by spectrophotometric method as described by Rhee and Kwon [9]. Initial rate was measured by finding the initial slope

of the plot of μM of free fatty acids produced versus time. Ratio of this initial rate and weight of the enzyme gives the activity.

Experimental Apparatus

Batch experiments were conducted in a glass stirred reactor of 120 mL capacity. The vessel has a jacket through which water at desired temperature was circulated.

Experimental Procedure

Deactivation kinetics data were obtained by subjecting the immobilized lipase enzyme preparation for denaturation under different temperature conditions without substrate. Brady *et al.* [10] had stated that the most of the lipases undergo rapid thermal deactivation at temperatures above 42°C. Hence, the present study was carried out at temperatures of 42°C and above.

The immobilized enzyme preparation suspended in 0.1 M phosphate buffer at pH 7.2 were incubated in the temperature range of 42°C to 55°C for five days. Lipase activity was assayed at 37°C and pH 7.2 for every 24 h time duration by using rice bran oil (50% v/v) as the substrate. The pH 7.2 was maintained by using 0.1 M phosphate buffer. The contents of the reactor were stirred by using a magnetic stirrer. Enzyme concentration was maintained at 11.45 U/mL. Stirring rate was kept at 200 rpm. Enzyme deactivation data were shown in the Fig. 1 and analyzed by integral method of analysis [11] for the first order deactivation model.

THEORETICAL CONSIDERATIONS

Based on the pseudo-steady state assumption the performance equation for PFR can be obtained as [2]

$$\tau = \frac{V}{v_0} = \frac{1}{(1-\varepsilon)} \int_{S_{in}}^{S_{out}} \frac{dS}{\eta(-r_s)} \tag{1}$$

Similarly performance eqn. for CSTR can be derived as

$$\tau = \frac{V}{v_0} = \frac{S_{in} \cdot x}{(1-\varepsilon)\eta(-r_s)} \tag{2}$$

In general volumetric reaction rate ($-r_s$) for enzymatic reaction can be written as

$$(-r_s) = E \cdot f(S,P) \tag{3}$$

The simplest expression of $f(S,P)$ is a hyperbolic function of S as given by Michaelis- Menten Equation,

$$f(S) = \frac{kS}{(K_m + S)} \tag{4}$$

Eqn. (4) has two extremities: (i) first –order kinetics $(-r_s) = [kE/K_m] \cdot S$ when $S \ll K_m$ and (ii) zero order kinetics, $(-r_s) = kE$ when $S \gg K_m$.

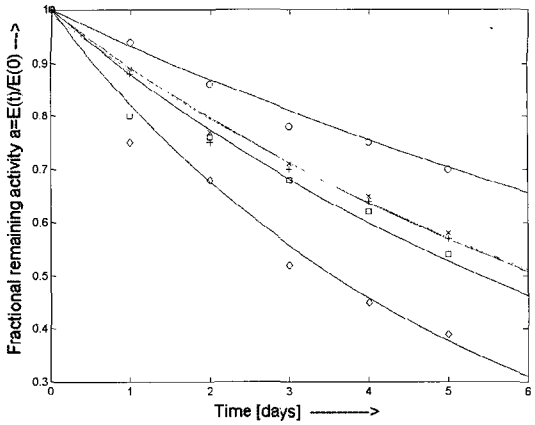

Fig. 1. Modeling of experimental deactivation data by using first- order deactivation model at various temperatures. Experimental data are shown by points: ○: 42°C, x: 45°C, +: 48°C, □: 52°C, ◇: 55°C. Solid lines for first order deactivation model.

Table 1. First order deactivation rate constants and standard deviations between model and experimental fractional remaining activities at various temperatures

Temperature (°C)	K_d (day^{-1})	σ
42	0.070	0.0169
45	0.113	0.0183
48	0.118	0.0170
52	0.128	0.0450
55	0.198	0.0504

$$\sigma = \left[\sum_{i=0}^{n} \{(a_{expi} - a_{modeli})/a_{expi}\}^2 / n \right]^{0.5}$$

Half-life ($t_{½}$) of Enzyme Activity

$$E(t) = \frac{E(0)}{2}; \text{ at } t = t_{½} \tag{5}$$

When the enzyme obeys first order decay model $E(t) / E(0)$ is reduced to

$$\frac{E(t)}{E(0)} = a = \exp(-k_d \cdot t) \tag{6}$$

Where '*a*' is the fractional remaining activity. Experimental data were fitted in this model and first order deactivation rate constants and standard deviations [12] at different temperatures were tabulated in Table 1.

No Limitation of Diffusion (η = 1.0) and Constant Feed Rate Policy

For PFR substitution of Eqn. (4) in Eqn. (1) and rearrangement gives

$$\frac{x(t) - k^1 \ln[1-x(t)]}{x(0) - k^1 \ln[1-x(0)]} = \frac{E(t)}{E(0)} = e^{-k_d t} \tag{7}$$

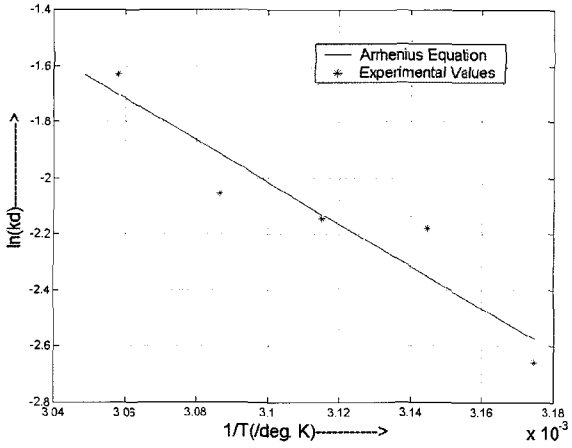

Fig. 2. Arrhenius plot for temperature dependency of deactivation rate. Experimental k_d values are shown by points. Solid line represents Arrhenius fit.

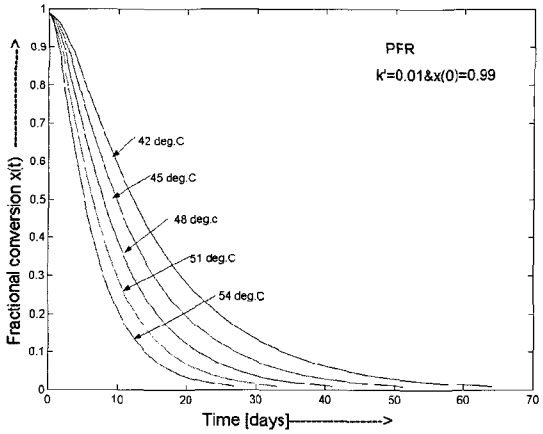

Fig. 3. Profile of decrease in $x(t)$ (in PFR) for Michaelis-Menten kinetics under constant feed rate policy. η = 1.0, k^1 = 0.01 and $x(0)$ = 0.99 parameter is temperature.

$$k^1 = K_m/S_{in}$$

Temperature dependency of deactivation rate constant is expressed by Arrhenius eqn.

$$k_d = A\ e^{\frac{-E^1}{RT}} \tag{8}$$

Where E^1 is the activation energy of the deactivation process and 'A' is the Arrhenius constant. Plot of $\ln(k_d)$ vs. 1/T was shown in Fig. 2.

Substituting Eqn. (8) in (7) we get for PFR :

$$\frac{x(t)-k^1\cdot\ln[1-x(t)]}{x(0)-k^1\cdot\ln[1-x(0)]}=\frac{E(t)}{E(0)}=e^{-A.e^{\frac{-E^1}{RT}}.t} \tag{9}$$

This equation is used for generation of time course $x(t)$ at different temperatures and at fixed k^1 and $x(0)$. At $x(0)$ = 0.99, k^1 = 0.01 plots were generated at different temperature for PFR by using Matlab Version 5.3 and shown in Fig. 3. Constant pH of 7.2 was assumed in PFR .

For CSTR substitution of Eqn. (4) in Eqn. (2) and rearrangement gives

$$\frac{x(t)+\dfrac{k^1x(t)}{[1-x(t)]}}{x(0)+\dfrac{k^1x(0)}{[1-x(0)]}}=\frac{E(t)}{E(0)}=e^{-A.e^{\frac{-E^1}{RT}}.t} \tag{10}$$

Profiles of $x(t)$ vs time were generated for CSTR by using Eqn. (10) and shown in Fig. 4.

Constant pH of 7.2 was assumed in CSTR.

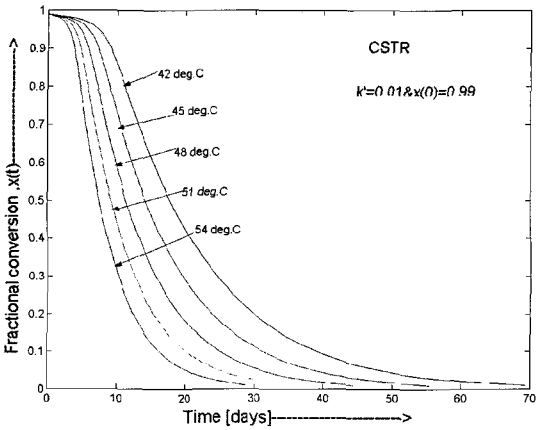

Fig. 4. Profile of decrease in $x(t)$ (in CSTR) for Michaelis-Menten kinetics under constant feed rate policy. η = 1.0, k^1 = 0.01 and $x(0)$ = 0.99 parameter is temperature.

Diffusion-influenced First Order Kinetics and Constant Feed Rate Policy

The overall rate of reaction of the immobilized enzyme particle is influenced by both intra particle diffusion and film around the particle. Only the intra particle diffusion-influenced case of the spherical particle is discussed here.

The effectiveness factor η of the first –order reaction in a spherical particle [13] is given by :

$$\frac{1}{\eta}=\frac{\varphi_1^2}{3(\varphi_1\coth\varphi_1-1)} \tag{11}$$

Where φ_1 is the thiele modulus of the first order kinetics of the sphere. The $\varphi_1$corresponding to

$$(-r_s)=\left(\frac{kE}{K_m}\right)\cdot S \text{ is}$$

$$\varphi_1=\frac{R_0}{3}\sqrt{\frac{(kE/K_m)\cdot\rho_p}{De}} \tag{12}$$

Then PFR performance Eqn. (1) becomes,

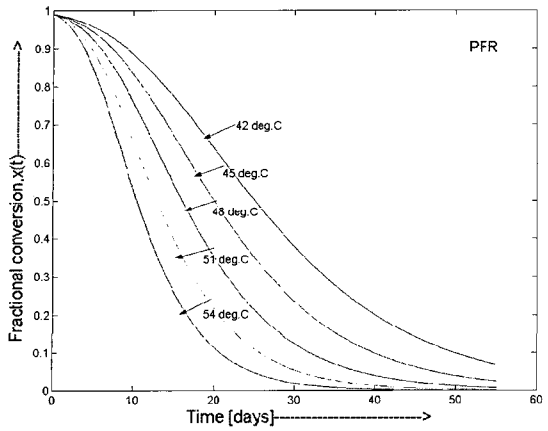

Fig. 5. Profile of decrease in $x(t)$ (in PFR) for first-order reaction when spherical immobilized particles used under constant feed rate policy. $\eta < 1.0$, $\varphi_1(0) = 0.5$ and $x(0) = 0.99$. Temperature is the parameter.

$$\tau = \frac{V}{v_0} = -\frac{R_0^2}{(1-\varepsilon)} \cdot \frac{1}{3[\varphi_1(t).\coth\varphi_1(t) - 1]} \cdot \ln[1-x(t)] \qquad (13)$$

From Eqn. (12)

$$\frac{\varphi_1(t)}{\varphi_1(0)} = \sqrt{\frac{E(t)}{E(0)}} \qquad (14)$$

When feed rate is constant during the continuous operation of PFR one gets the following expression from Eqn. (13).

$$\frac{\ln[1-x(t)]}{\ln[1-x(0)]} = \frac{\varphi_1(0).\sqrt{\frac{E(t)}{E(0)}}\coth[\varphi_1(0)\sqrt{e^{-A.e^{-E/RT}t}}] - 1}{\varphi_1(0).\coth\varphi_1(0) - 1} \qquad (15)$$

The profiles of time course $x(t)$ by using Eqn. (15) for PFR at different temperatures when $\varphi_1(0) = 0.5$ and $x(0) = 0.99$ were generated and shown in Fig. 5.

The first-order reaction influenced by intra particle diffusion in the CSTR is given by

$$\tau = \frac{V}{v_0} = \frac{R_0^2}{(1-\varepsilon)De} \frac{1}{3[\varphi_1(t) \cdot \coth\varphi_1(t) - 1]} \cdot \frac{x(t)}{[1-x(t)]} \qquad (16)$$

When the constant feed rate policy is adopted during the continuous operation, one gets the following expression from Eqn. (16):

$$\frac{\frac{x(t)}{[1-x(t)]}}{\frac{x(0)}{[1-x(0)]}} = \frac{\varphi_1(0).\sqrt{\frac{E(t)}{E(0)}}\coth[\varphi_1(0)\sqrt{\frac{E(t)}{E(0)}}] - 1}{\varphi_1(0) \cdot \coth\varphi_1(0) - 1} \qquad (17)$$

For first order deactivation model with Arrehenius temperature dependecy of deactivation rate constant, Eqn. (17) becomes:

$$\frac{\frac{x(t)}{[1-x(t)]}}{\frac{x(0)}{[1-x(0)]}} = \frac{\varphi_1(0) \cdot \sqrt{e^{-A.e^{-E/RT}t}}\coth[\varphi_1(0)\sqrt{e^{-A.e^{-E/RT}t}}] - 1}{\varphi_1(0) \cdot \coth\varphi_1(0) - 1} \qquad (18)$$

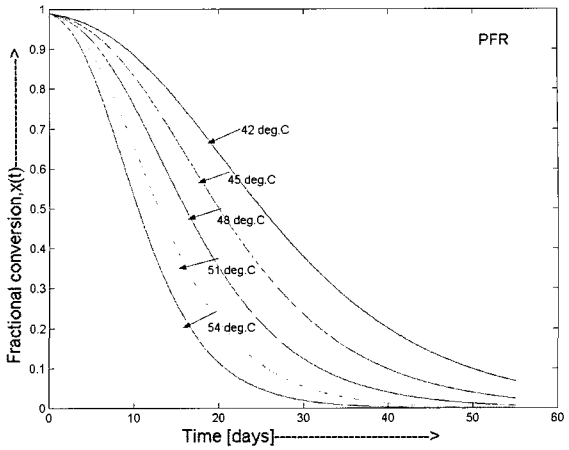

Fig. 6. Profile of decrease in $x(t)$ (in CSTR) for first-order reaction when spherical immobilized particles used under constant feed rate policy. $\eta < 1.0$, $\varphi_1(0) = 0.5$ and $x(0) = 0.99$. Temperature is the parameter.

The profiles of $x(t)$ versus time by using Eqn. (18) for CSTR at different temperatures were generated when $\varphi_1(0) = 0.5$, $x(0) = 0.99$ and shown in Fig. 6.

RESULTS AND DISCUSSION

Experimental deactivation data were well fitted by first order deactivation model with an average standard deviation of 0.0295. Higher deactivation rates have been observed at higher temperatures. The temperature dependency of deactivation rate is well represented by Arrhenius equation with standard deviation of 0.1332. From the plot of $\ln(k_d)$ vs $1/T$, the activation energy for thermal deactivation process was found to be 14.89 kcal/mol.

The time course of conversion due to enzyme deactivation was predicted for various situations and shown in Fig. 3-6. The study predicts apparent half-lives of 254 h and 187 h at 40°C and 44°C respectively for PFR in no diffusion control region when $k^1=0$ (Eqn. (7)). Kenneth *et al.* [6] had reported the half-life of immobilized lipase enzyme in the hollow fibre reactor as 170 h at 40°C and a buffer pH of 7.0. Garcia *et al.* [7] had reported a half-life of 96.7 h for the same enzyme immobilized on microporous hollow fiber at 44°C, pH 7.0. The deviation between predicted half-lives and actual half-lives is found to increase as the k^1 increases.

Considerable increase in the operation stability of the immobilized lipase enzyme is observed with decrease in temperature. Higher apparent half-lives are predicted in the case of CSTR than PFR.

Table 2 represents the effect of $x(0)$ on apparent half-life during constant feed rate policy in no diffusion control region for Michaelis-Menten kinetics. The apparent half-life decreases as $x(0)$ decreases. Similar observations are noticed for diffusion control region of first order reaction and were shown in Table 3.

Table 2. Comparision of apparent half-lives of spherical immobilized enzyme particles under constant feed rate policy for Michaelis –Menten kinetics and for the case of $x(0) = 0.99$ and $x(0) = 0.9$ (For no diffusion limitation region $\eta = 1.0$, and $k^1 = 0.01$)

	PER					CSTR				
X(0)	42°C	45°C	48°C	51°C	54 °C	42°C	45°C	48°C	51°C	54°C
0.99	10.0	7.72	6.36	5.0	3.60	17.72	14.09	11.36	9.55	7.72
0.90	8.18	5.91	5.0	4.09	3.41	9.0	7.25	5.5	4.5	4.0

Table 3. Comparision of apparent half-lives of spherical immobilized enzyme particles under constant feed rate policy for Michaelis –Menten kinetics and for the case of $k^1 = 0.01$ and $k^1 = 0.1$ (For no diffusion control region $\eta = 1.0$, and $x(0) = 0.99$)

	PER					CSTR				
k^1	42°C	45°C	48°C	51°C	54°C	42°C	45°C	48°C	51°C	54°C
0.01	10.0	7.72	6.36	5.0	3.6	17.72	14.09	11.36	9.55	7.72
0.10	12.27	10.0	8.18	6.36	4.55	36.87	30.0	21.56	18.75	15.31

Table 4. Comparison of apparent half-lives of spherical immobilized enzyme particles under constant feed rate policy for first order reaction and for the case of $x(0) = 0.99$ and $x(0) = 0.90$ (For diffusion control region $\eta < 1.0$, and $\varphi_1(0) = 0.5$)

	PER					CSTR				
X(0)	42°C	45°C	48°C	51°C	54°C	42°C	45°C	48°C	51°C	54°C
0.99	25	20	16.15	12.69	10.0	59.37	46.87	37.50	30.62	25.0
0.90	15.57	12.31	10.0	8.26	6.73	28.13	22.50	18.12	13.75	10.62

Table 5. Comparison of apparent half-lives of spherical immobilized enzyme particles under constant feed rate policy for first order reaction and for the case of and $\varphi_1(0) = 0.5$, $\varphi_1(0) = 5$ (For diffusion control region $\eta < 1.0$, and $x(0) = 0.99$)

	PER					CSTR				
$\varphi_1(0)$	42°C	45°C	48°C	51°C	54°C	42°C	45°C	48°C	51°C	54°C
0.5	25.0	20.0	16.15	12.69	10.0	59.37	46.87	37.50	30.62	25.0
5.0	36.53	26.15	20.77	16.92	13.46	66.88	53.13	43.13	34.37	27.50

Effect of initial substrate concentration or k^1 is shown in Table 4. The apparent half-lives decrease as the initial substrate concentration increases or k^1 decreases. This may be due to the inhibitory action of substrate on enzyme active sites.

Effect of initial thiele modulus on apparent half-life is shown in Table 5. Apparent half-lives increase as the initial thiele modulus increases. Initial thiele modulus for immobilized enzyme preparation depends on initial enzyme activity and support particle characteristics (Eqn. (12)). For a given particle it only depends on initial enzyme activity. The study predicts enzymes with high initial activity will have better stability.

CONCLUSION

The study shows that lipase is unstable at higher temperatures. 64% of the original activity is lost due to thermal deactivation when immobilized enzyme preparation was incubated at 55°C for 5 days. Due consideration should therefore be given to the effect of temperature on thermal deactivation of lipase enzyme as it adversely affects the bioprocess overall economy.

In no diffusion control region apparent half-lives are influenced by initial conversion level and initial substrate concentration, where as in diffusion control region initial thiele modulus is found to influence the apparent half-life apart from the initial conversion levels. Higher half-lives are predicted as the system tends towards diffusion control region. Apparent half-life used in our present study is not the real half-life of enzyme. However, apparent half-life can be used as an indirect measure to predict the stability of lipase enzyme in the continuous process.

NOMENCLATURE

a_{expi}	Fractional remaining activity from experiments at i th day (dimensionless)
a_{modeli}	Fractional remaining activity from first order deactivation model at i th day (dimensionless)
CSTR	Continuous stirred tank reactor
D_e	Effective diffusivity (m^2/sec)
E	Enzyme concentration (U/m^3)
E'	Activation Energy of the deactivation process
FFA	Free fatty acid
$f(S)$,$f(S,P)$	Functions of S and functions of S and P.
k_d	First order deactivation constant (day^{-1})
K_m	Michaelis constant (mol/L)
k^1	K_m/S_{in} (dimensionless)
n	Number of experimental points (dimensionless)
PFR	Plug flow reactor
R_0	Radius of spherical particle (m)
r_s	Volumetric reaction rate (mol L^{-1} sec^{-1})
S	Substrate concentration (mol/L)
S_{in} , S_{out}	Inlet and out let substrate concentrations (mol/L)
T	Temperature (°K)
t	Time (sec)
v_0	Volumetric feed rate of substrate solution (m^3/sec)
V/v_0	Space velocity (sec^{-1})
V	Working volume of bioreactor (m^3)
x	Conversion =1- S_{out}/S_{in} (dimensionless)
$x(t)$	Time course conversion due to enzyme deactivation (dimensionless)
$x(0)$	Initial conversion when enzyme activity is $E(0)$.

Greek Letters

ε	Void fraction (dimensionless)
τ	Space time (s)
σ	Standard deviation between experimental and model values (dimensionless)
η	Effectiveness factor of immobilized enzyme particle (dimensionless)
φ_1	$R(kE/K_mD_e)^{1/2}$ Thiele modulus of first-order reaction in sphere (dimensionless)
$\varphi_1(0)$	$R\{kE(0)/K_mD_e\}^{1/2}$, φ_1 at time 0 (dimensionless)
$\varphi_1(t)$	$R\{kE(t)/K_mD_e\}^{1/2}$, φ_1 at time t (dimensionless)

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