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Performance of Fixed and Fluidized Bed Reactors with Immobilized Enzyme

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ABSTRACT

Saccharification of α-amylase liquefied cassava starch was carried out at pH 4.5 and 45°C, in both fixed and fluidized bed reactors, using amyloglucosidase immobilized in 0.5 mm controlled pore silica particles. Reactor performance data are compared with mathematical modeling. Data show that for equal normalized residence time and lower fluid-bed porosities, the fluidized bed mode leads to higher conversions than fixed bed. Interparticle mass transfer experiments showed absence of diffusion limitations and starch conversion reached 98.5% with a real residence time of only 10 min, whereas the conventional liquid-phase process requires 48 h to achieve the same conversion.

Index Entries: Amyloglucosidase; immobilized enzyme; cassava starch; fluidized bed; controlled pore silica.

NOMENCLATURE

A_i	immobilized enzyme activity, U/g
C_{Ao}	initial starch concentration, 300 mg/mL
C_g	glucose concentration, mg/mL
C_{gi}	initial glucose concentration, mg/mL
d_i	internal reactor diameter, 6.88 mm
D	diffusivity of average size maltodextrin, $D = 4.49 \cdot 10^{-6}$ cm²/s (*8*)
d_p	particle mean diameter, 0.463 mm

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E_T	total enzymatic activity, $E_T = M_e A_i$, U
f	ratio of molecular weights for the anhydroglucose unit in the starch molecule and glucose, $f = 162/180 = 0.9$
I.E.	immobilized enzyme
k_c	external mass-transfer coefficient, cm/s
K_i	product inhibition constant, g/L
K_m	Michaelis-Menten constant, g/L
K_s	substrate inhibition constant, g/L
k_{cat}	reaction rate constant related to product formation, g/min·U
M_e	immobilized enzyme mass, dry weight, g
m	parameter in Eq. (8)
n	Richardson and Zaki expansion coefficient
r	coefficient of correlation
Re	particle Reynolds number, $Re = \rho u d_p / \mu$
Sc	Schmidt number, $Sc = \mu / \rho D$
t_R	fluid real residence time, $t_R = V_T \epsilon / v$, min
u	superficial fluid velocity, cm/s
U_{mf}	minimum fluidization velocity, cm/s
U_t	particle terminal velocity, $\log U_t = \log U_{tc} + d_p/d_i$, cm/s
U_{tc}	corrected particle terminal velocity, cm/s
V_L	reactor liquid volume, $V_L = M_e \epsilon / [(1 - \epsilon) \rho_p]$, cm^3
V_T	total reactor volume, cm^3
v	volumetric flow rate, cm^3/s
X_A	conversion of starch to glucose, %
α, β, γ	parameters in Eq. (1), defined in Eqs. (2)–(4)
α_1	parameter in Eq. (8)
ϵ	bed porosity
ϵ_p	particle porosity, $\epsilon_p = 0.568$
μ	viscosity of the liquefied starch solution (30% w/v, 45°C), $\mu = 2.34 \cdot 10^{-2}$ g/cm s (*8*)
ρ	density of the liquefied starch solution (30% w/v, 45°C), $\rho = 1.101$ g/cm^3 (*8*)
ρ_p	particle density, $\rho_p = 0.939$ g/cm^3 (*8*)
ρ_s	particle matrix density, $\rho_s = 2.714$ g/cm^3 (*8*)
τ_p	normalized residence time, $\tau_p = E_T t_R / V_L$, U·min/cm^3

INTRODUCTION

The objectives of this work were to compare the performance of fixed and fluidized bed reactors for the saccharification of cassava starch and to develop reactor modeling. Reactor bed was made of immobilized enzyme particles for which the fluidization characteristics and the existence of interparticle diffusion limitations were examined. Intraparticle diffusion limitations are not expected owing to the large and regular particle pores.

REACTOR MODELING

The hydrolysis of starch is usually accomplished in two steps. In the first step, called liquefaction, starch is thoroughly depolymerized with α-amylase producing various maltodextrin intermediates and a small proportion of glucose. In the second step, called saccharification, amyloglucosidase (AMG) acts on these intermediates producing glucose and dextrines of lower molecular weight. Rigorous modeling of starch saccharification becomes very complex owing to the competition among these various dextrines for the catalytic sites of AMG (*1,2*).

Starch conversion (X_A) as a function of superficial velocity was adjusted by a single substrate model where all dextrines were grouped in a single substrate (*3–6*). It is assumed that starch saccharification, in both fixed and fluidized bed reactors, can be represented by Michaelis-Menten kinetics added with substrate (K_s) and product (K_i) inhibition. A material balance in an ideal tubular plug flow reactor (*4–8*) yields the integrated equation:

$$\tau_p = \alpha X_A - \beta \ln(1 - X_A) - \gamma X_A^2 \quad (1)$$

where:

$$\alpha = (C_{Ao}/k_{cat})\,(1 - K_m/K_i + C_{Ao}/K_s) \quad (2)$$

$$\beta = (K_m/k_{cat})\,(1 + C_{gi}/K_i + C_{Ao}/K_i) \quad (3)$$

$$\gamma = C_{Ao}^2/(2\,K_s\,k_{cat}) \quad (4)$$

$$\tau_p = E_T/v \quad (5)$$

$$X_A = 100\,f\,(C_g - C_{gi})/(C_{Ao} - f\,C_{gi}) \quad (6)$$

MATERIALS AND METHODS

Enzyme

Aspergillus niger amyloglucosidase was supplied by NOVO (AMG 150 L) and immobilized using the glutaraldehyde method (*9*) as adapted in our previous work (*10*). The protein load was 17.3 mg/g of suport and the activity of the immobilized enzyme was 676.8 U/g of dry support. Each unit corresponds to the production of 1 μmol of glucose/min at 45°C, and with 30% (w/v) starch solution, pH 4.5, as substrate.

Support

Controlled pore silica (CPS) was supplied by Corning Glass Works (Corning, NY), with a nominal size of 0.5 mm. Average pore diameter was 37.5 nm. Other particle properties are given together with the nomenclature.

Substrate

The substrate was α-amylase liquefied cassava starch (COPAGRA-PR) at a concentration of 30% (w/v), pH 4.5, 45°C. Details of the liquefaction method are reported in previous work (*10*).

Fixed and Fluidized Bed Reactors

Reactor column was made of glass with an internal diameter (d_i) of 6.88 mm and a maximum useful height of 1200 mm. It was operated with down and upward liquid flow giving fixed and fluidized beds, respectively. The liquid distributor was a stainless steel mesh pressed in spherical form. Isothermicity was assured by recirculating water at constant temperature in the reactor jacket.

Glucose Assay

Glucose produced by saccharification was assayed by the orthotoluidine method (*11*).

Fluidization Parameters

To determine the fluidization parameters, 11.64 g of CPS were placed inside the reactor and the liquefied starch flow rate was gradually increased until the maximum bed height was reached. Subsequently, the liquid superficial velocity (u) was slowly reduced in steps and kept constant for 20 min at each step. The expanded bed height and fluid flow rate were then measured for each step. The bed porosity (ϵ) and minimum fluidization velocity (U_{mf}) were then obtained from these data with standard procedures (*12–14*).

Conversion Tests

These tests were carried out by loading the I.E. into the reactor in successive additions of approx 1.5 g (dry basis) until the maximum reactor height was reached. After each addition, the liquid flow was resumed and 10–30 min were allowed for reaching steady state. The conversion was determined at regular intervals by sampling the reactor outlet and assaying for glucose. The reactor was operated first as a fluidized bed and then as a fixed bed. Detailed experimental conditions are listed in Table 1.

Table 1
Conversion Tests

Variables	Fluidized bed			Fixed bed		
Superficial liquid velocity (u, cm/s)	0.101	0.148	0.323	0.099	0.143	0.323
Bed porosity (ϵ)	0.556	0.594	0.711	0.410	0.413	0.433
mg of enzyme/cm^3 of reactor	7.2	6.6	4.7	9.6	9.5	9.2
Number of I.E. additions	12	10	8	12	10	8
Time interval allowed for steady state (min)	10	10	10	30	30	20
Number of conversion measurements after steady state	6	6	6	10	10	9
Time interval between conversion measurements (min)	10	10	10	10	10	10
Total time for conversion measurements (h)	12	10	8	24	20	13.3
Final conditions						
Bed height (cm)	110	103	112	87	72	58
Approximate total I.E. mass (M_e, g)	18	15	12	18	15	12
Real residence time, (t_R, min)	10.0	6.9	4.1	6.4	3.5	1.2
Conversion (X_A, %)	98.5	71.7	34.3	80.9	68.1	33.4

Table 2
Interparticle Mass Transfer Test

Experimental values					Calculated	
Me (g)	*u* (cm/s)	M_e/u	t_R (min)	X_A (%)	k_c (cm/s) Eq. (9)	t_R (min) Eq. (10)
2.0029	0.1550	12.92	0.355	20.2	0.00295	0.00984
4.0059	0.3151	12.71	0.403	20.0	0.00374	0.00767
6.0088	0.4700	12.78	0.411	19.7	0.00427	0.00661
8.0118	0.6250	12.82	0.433	19.9	0.00469	0.00608
10.0147	0.7890	12.69	0.434	17.9	0.00507	0.00500
12.6067	0.9311	12.95	0.415	18.0	0.00536	0.00476

Interparticle Mass Transfer Test

In this test the conversion was measured for different superficial velocities in the fixed bed reactor. For each experimental condition the liquid velocity and I.E. charged into the reactor were simultaneously increased in the same proportion so as to maintain the ratio M_e/u constant. The objective of the test was to produce increasingly higher particle Reynolds numbers while keeping the fluid residence time constant. Therefore, if a constant conversion is obtained, the interparticle diffusion limitations are negligible (*15*). Experimental conditions are shown in Table 2.

RESULTS AND DISCUSSION

Fluidization Parameters

Fluidized bed expansion data shown in Fig. 1 were fitted to the Richardson-Zaki correlation (*13*), $u/U_{tc} = \epsilon^n$, giving:

$$u = 2.57\,\epsilon^{5.82},\ r = 0.9992 \qquad (7)$$

Experimental fluidization parameters, U_{mf}, n, and U_t, obtained from Eq. (7) are 0.0213, 5.82, and 3.00 cm/s. Calculated values, obtained from established correlations (*12–14*) are 0.0245, 4.80, and 2.42 cm/s. Experimental and calculated values are in moderate agreement. However, the discrepancies observed are within the usual standard deviation of the available correlations, which for U_{mf} reaches ±34% (*12*). For greater accuracy, it is advisable to determine these parameters experimentally. Equation (7) also was used to calculate the liquid velocity required to obtain desired bed porosity in the conversion test.

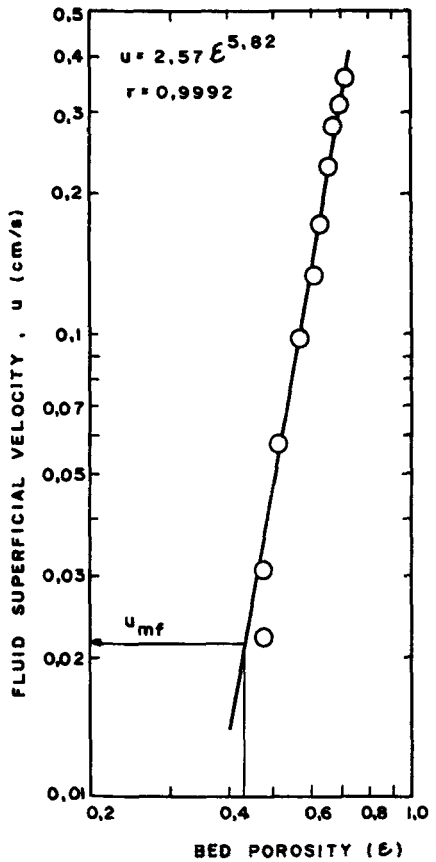


Fig. 1. Relation betwen the liquefied starch superficial velocity (u) and bed porosity (ϵ) for the fluidized bed reactor.

Interparticle Mass Transfer

Conversion and mass transfer coefficient as a function of superficial liquid velocity are presented in Table 2 and Fig. 2. The mass transfer coefficients were calculated with the correlation of Nelson and Galloway (*16*) as modified by Rowe (*17*) and Koloini et al. (*18*) for a fluidized bed:

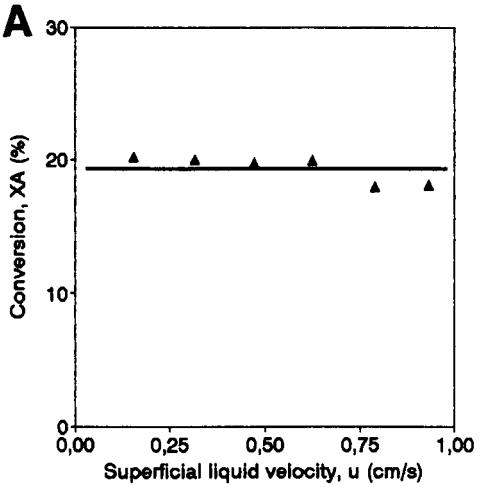
$$k_c = (D\,Sc^{2/3}/d_p\,\epsilon)\,\{\epsilon^{1-2m}\,[1/(1-\epsilon)^{1/3} - 1]\,\alpha_1^2\,Re\} / [2(1-\epsilon)^{1/3}] \qquad (8)$$

with $\alpha_1 = 0.7$, $Re < 2$, $m = 0.5$.

The correlation of Wilson and Geankoplis (*19*):

$$k_c = 1.09\,u / [(Re\,Sc)^{2/3}\,\epsilon],\ 0.0016 < Re < 55 \qquad (9)$$

was utilized for the fixed bed. These data show a nearly constant conversion despite the almost 10-fold increase in fluid velocity. This result demonstrates a negligible interparticle mass transfer resistance and is in line with Marsh et al. (*20*).



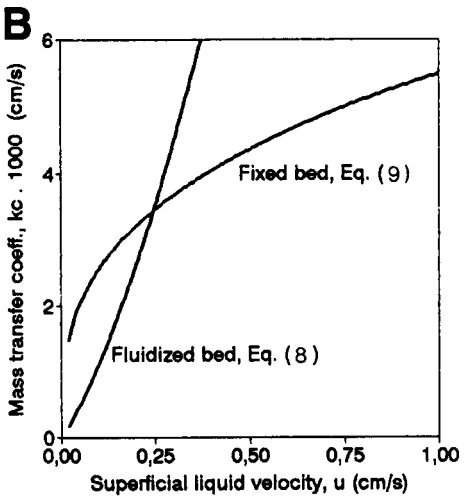


Fig. 2. Conversion (X_A) and mass transfer coefficient (k_c) as a function of the fluid superficial velocity (u).

If interparticle mass transfer was the rate limiting step then the residence time needed to achieve a given conversion would be (*21*):

$$t_R = -(d_p/6\,k_c)\ln(1 - X_A) \qquad (10)$$

As shown in Table 2 the residence time calculated with Eq. (10) is many times smaller than the experimental value and therefore this criterion corroborates the absence of interparticle mass transfer limitations.

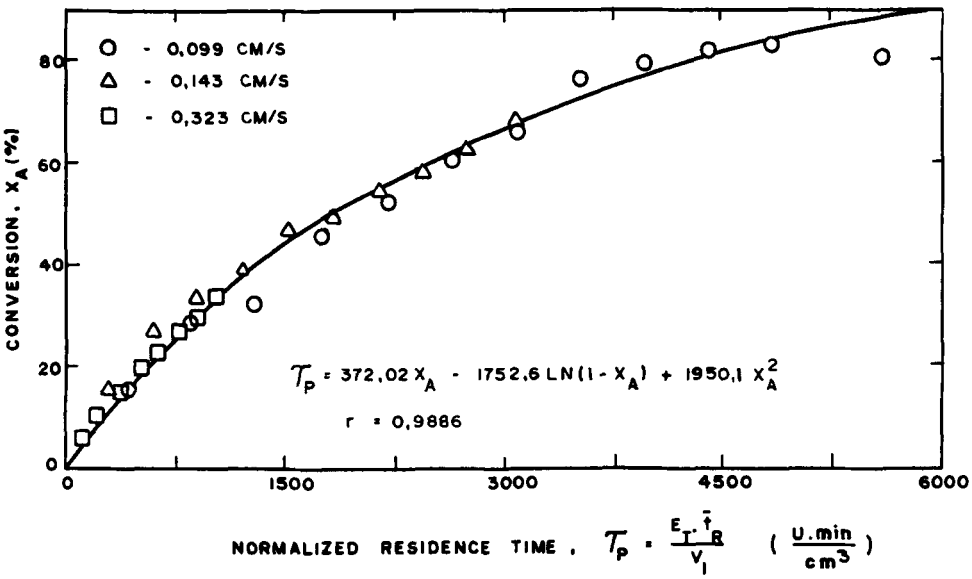


Fig. 3. Conversion (X_A) as a function of the normalized residence time (τ_p) for the fixed bed reactor.

Reactor Modeling

Experimental results for starch conversion (X_A) as a function of normalized residence time (τ_p) are presented in Figs. 3 and 4 for fixed and fluidized bed, respectively. The curves shown are drawn with Eq. (1) adjusted to these data. Resulting parameters α, β, and γ are also given.

Figure 3 shows that starch conversion as a function of τ_p for the fixed bed reactor, at all superficial velocities, collapses to a single curve, which is well correlated by the single substrate model. Conversion-residence time data for the fluidized bed were also satisfactorily correlated by the single substrate model as demonstrated in Fig. 4. Analysis of the adjusted parameters, however, reveals a negative value for γ, which in turn implies a negative reaction velocity constant, k_{cat} in Eq. (4). This is a result that is not physically meaningful and suggests the need to improve modeling. Cardoso (*6*) has recommended that, for practical purposes of design, this model's utility is its simplicity and capability of fitting conversion data. This way, lab scale experiments can be simply scaled-up to industrial applications. The limitation of this approach is that the parameters α, β, and γ cannot be calculated from knowledge of the kinetic constants and Eqs. (2–4).

Since internal and external diffusion limitations are negligible, improvement in modeling cannot be obtained by inclusion of mass transfer considerations but by recapturing the complexities of liquid starch saccharification. These complexities include the formation of various intermediate

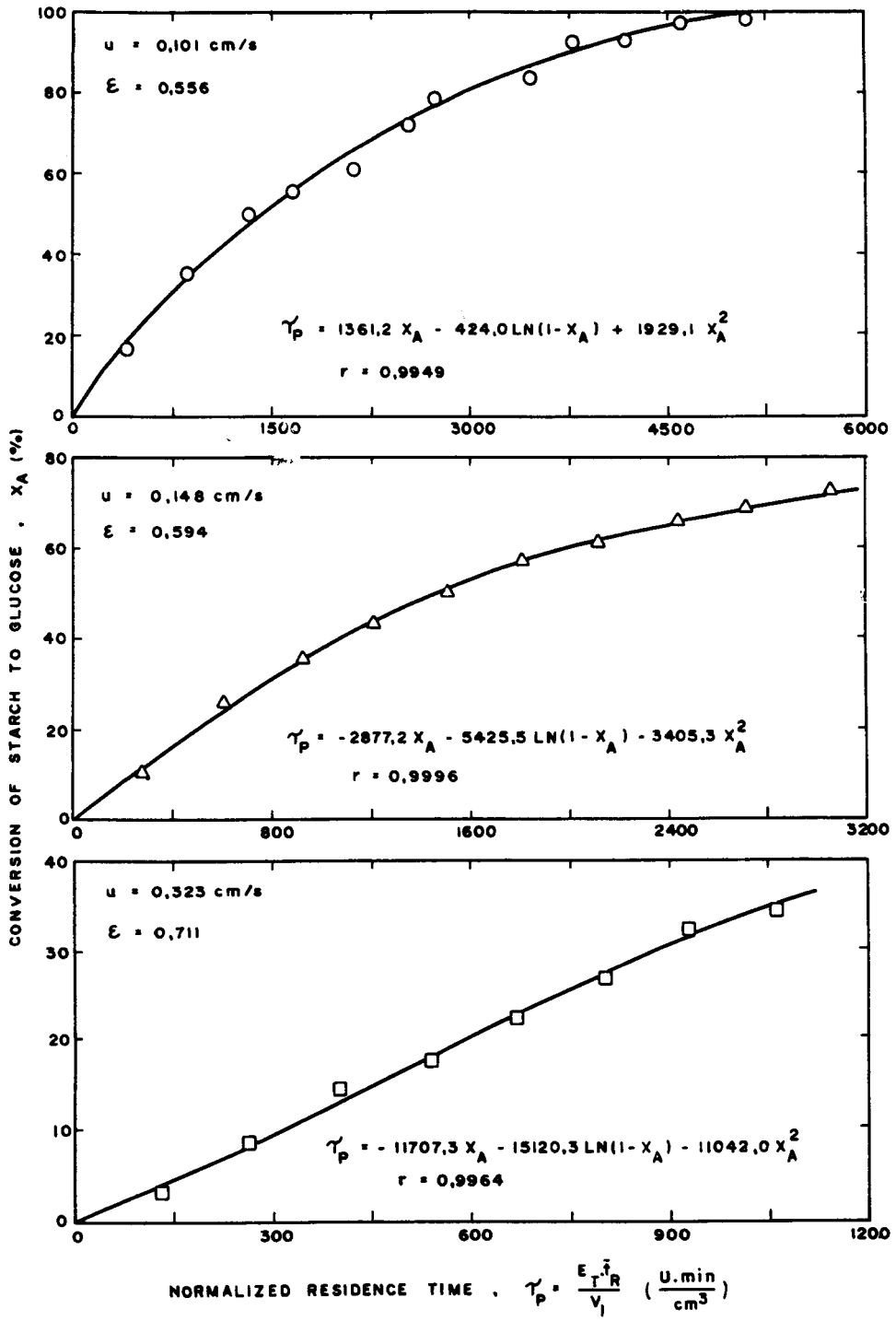
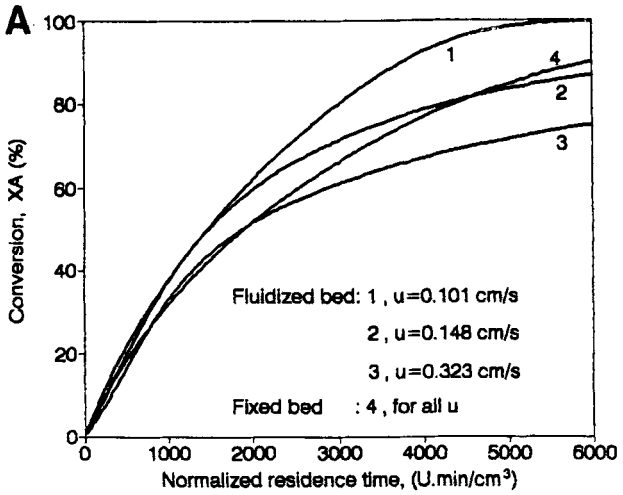
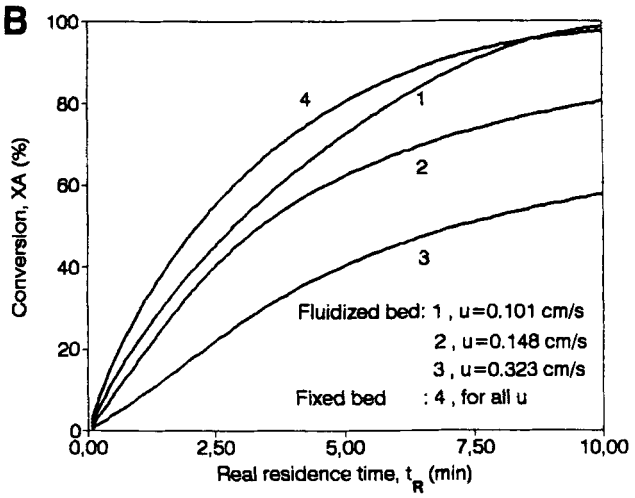


Fig. 4. Conversion (X_A) as a function of the normalized residence time (τ_p) for the fluidized bed reactor.



Conversion as a function of normalized residence time



Conversion as a function of real residence time

Fig. 5. Comparison of fixed and fluidized bed reactor performance for a given real or normalized residence time (t_R or τ_p). Equation (1) and the parameters of Figs. 3 and 4 were used for drawing the performance curves.

substrates referred above. This approach was followed by Zanin (*8*) and details are currently being prepared for publication.

Fixed Vs Fluidized Bed

Figure 5A shows that at the same τ_p, which is equivalent to the same I.E. mass and flow rate, the fluidized bed reactor has a better performance than the fixed bed if the liquid superficial velocity is low. However, this

trend reverses for the higher superficial velocities, at conversion values that depend on bed expansion. The higher performance of the fluidized bed, also observed by others (4,6,22), has been explained before through higher mass transfer. Figure 2 shows that for $u < 0.25$ cm/s, k_c is smaller for the fluidized bed, whereas according to the above interpretation it should be greater. Moreover, mass transfer limitations were shown above to be negligible anyway. Therefore, an alternative explanation is required. Lower performance of the fixed bed may be owing to fluid stagnation at interparticle contact points and channeling. Reversion of this trend observed at higher superficial velocities may arise from particle recirculation in the fluidized bed and less efficient liquid-particle contact patterns.

If both reactors are now compared at the same real residence time (Fig. 5B), the fixed bed presents, in general, higher performance. This difference increases for higher superficial velocities associated with higher bed expansions in the fluidized bed. The explanation is that at the same real residence time the fluid meets more I.E. inside the fixed bed than in a fluidized bed, because the former has always a lower bed porosity. See in Table 2 the entry for mg of enzyme/cm^3 of reactor volume.

Free Vs Immobilized Enzyme Technology

As shown in Table 1 it was possible to achieve starch conversion of 98.5% in a real residence time of only 10 min operating with fluidized bed. In the conventional liquid-phase process, a minimum period of 48 h is normally required to obtain equivalent conversion. Contact time reduction results from a much greater enzyme concentration achieved in the fluidized bed, 7.2 mg of enzyme/cm^3 of reactor volume (Table 2) against 0.045 for the conventional process. The ratio of the reactor volumes needed in these two alternatives, $V_{\text{T-conventional}}/V_{\text{T-I.E.}}$, is (conventional space time $\cdot v$)/($t_R\ v/\epsilon$) or about 160, giving a great advantage for the immobilized enzyme process. Deactivation of the immobilized enzyme during operation lowers this advantage. However, even if owing to economic reasons the I.E. charge in the fluidized bed has to be used for two half-lives the reactor volume ratio is still 40.

CONCLUSIONS

The conclusions reached in this work are:

1. A moderate agreement is obtained between experimental and calculated fluidization parameters. Hence if higher precision is required, experimental determination is recommended.
2. The interparticle mass transfer resistance for the superficial velocities of this study is negligible.

3. For the fixed bed reactor, the conversion-residence time data obtained at all flow rates could be represented by a single curve, which is satisfactorily adjusted by the single substrate model.
4. Although adjusted parameters reveal inconsistency of fundamental nature in this model, the fitted equation can be used with objective of reactor design.
5. Conversion-residence time results with fluidized bed are also satisfactorily fitted by the single substrate model.
6. Given the same quantity of immobilized enzyme, higher conversions are obtained with the fluidized bed reactor at smaller superficial velocities associated with lower expansions.
7. Given the same real residence time, the fixed bed reactor leads, in general, to higher conversions.
8. With the fluidized bed reactor, a conversion of 98.5% was obtained in a real residence time of only 10 min, which implies a reactor many times smaller than that utilized in the conventional process of starch saccharification.

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