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Modeling Fixed and Fluidized Reactors for Cassava Starch Saccharification with Immobilized Enzyme

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ABSTRACT

Cassava starch saccharification in fixed-and fluidized-bed reactors using immobilized enzyme was modeled in a previous paper using a simple model in which all dextrins were grouped in a single substrate. In that case, although good fit of the model to experimental data was obtained, physical inconsistency appeared as negative kinetic constants. In this work, a multisubstrate model, developed earlier for saccharification with free enzyme, is adapted for immobilized enzyme. This latter model takes into account the formation of intermediate substrates, which are dextrins competing for the catalytic site of the enzyme, reversibility of some reactions, inhibition by substrate and product, and the formation of isomaltose. Kinetic parameters to be used with this model were obtained from initial velocity saccharification tests using the immobilized enzyme and different liquefied starch concentrations. The new model was found to be valid for modeling both fixed- and fluidized-bed reactors. It did not present inconsistencies as the earlier one had and has shown that apparent glucose inhibition is about seven times higher in the fixed-bed than in fluidized-bed reactor.

Index Entries: Cassava starch; amyloglucosidase; immobilized enzyme; fluidized bed; controlled pore silica.

INTRODUCTION

In a previous paper *(1)*, the performance of fixed- and fluidized-bed reactors with immobilized amyloglucosidase was examined for the purpose of saccharifying liquefied cassava starch. Data showed that for equal

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normalized residence time (enzyme activity per volume multiplied by real residence time) and lower fluid-bed porosities, the fluidized-bed reactor led to higher conversion than fixed bed. The immobilized-bed enzyme (IE) support particle was controlled pore silica (CPS) with 0.5-mm particle diameter and an average pore diameter of 37.5 nm. Interparticle mass transfer experiments showed absence of diffusion limitations, and reactor conversion was modeled by grouping all intermediate dextrins of starch hydrolysis in a single substrate model, which allowed for substrate and product inhibition. Taking the fixed- and fluidized-bed systems as plug flow reactors, this model yielded the simple integrated Eq. (1) of ref. *(1)*.

Conversion-residence time data for both fixed- and fluidized-bed reactors were satisfactorily fitted by this equation. Analysis of the adjusted parameters, however, revealed a negative value for the reaction velocity constant that is not physically meaningful. This result suggested the need for improvements in reactor modeling.

A more realistic cassava starch hydrolysis model was developed and applied initially to a batch reactor containing free enzyme *(2)*. This is a multisubstrate model that considers intermediate dextrins, reversibility of some reactions, substrate and product inhibition, in addition to allowing for isomaltose synthesis from glucose, and competition among dextrins for the active site of amyloglucosidase. This model does not present the aforementioned inconsistency of negative kinetic constants, and showed good agreement with the batch reactor data in the presence or absence of added glucose. Although still relatively easy to use, this model is mathematically more complex than the earlier one, and requires the solution of six ordinary differential equations coupled to five algebrical relations *(2,3)*.

In this article, the later model *(2)* will be adapted for saccharification of cassava starch in fixed- and fluidized-bed reactors of immobilized amyloglucosidase. The earlier data *(1)* will be compared to modeling.

MATERIALS AND METHODS

To avoid extensive repetition, reference is made to information contained in the previous paper *(1)*. There are descriptions to be found of the Enzyme, Substrate, Reactors, Assay Methods, and Conversion Tests.

In addition, described here are the tests used to determine:

1. The influence of immobilized enzyme mass on initial starch hydrolysis rate;
2. Intraparticle diffusion limitations; and
3. Kinetic parameters observed with the immobilized enzyme.

The IE used inside the reactors and in test 2 had a higher amyloglucosidase load (17.3 mg/g_{IE}) and activity (676.8 U/g_{IE}) *(1)*, whereas the IE

used with tests 1 and 3 had a load of 4.12 mg/g_{IE} and activity equal to 188.5 U/g_{IE}. Each enzyme unit corresponds to the quantity of enzyme that produces 1 μmol of glucose/min at 45°C, pH 4.5, and with 30% (w/v) liquefied cassava starch as substrate.

Influence of Immobilized Enzyme Mass on Initial Starch Hydrolysis Rate

Since the kinetic parameters to be found for the immobilized enzyme will be determined in a batch microreactor containing a small basket where the IE is retained, it was necessary to know up to what mass of IE could be used inside the basket before reaction limitations would cause the initial hydrolysis rate to deviate from being linearly proportional to the quantity of enzyme used.

This test was conducted with 50 mL of liquefied starch solution, 1% (w/v) at 45°C, and pH 4.5. Immobilized enzyme mass loaded into the basket varied from 0.08–1.0 g, dry wt. Starting from time zero when the loaded basket was dipped into the starch solution, samples were taken at regular intervals of 2 mins. These samples were boiled for 10 min and kept at 4°C until assayed for glucose produced. A linear fit of micromoles of glucose produced vs time gave the initial rate of liquefied starch hydrolysis.

Intraparticle Diffusion Limitations

Arrhenius plots (log of initial rate of hydrolysis reaction vs the inverse of absolute temperature) obtained for the soluble and immobilized enzyme, at the same substrate concentration, allow inference of the presence of intraparticle diffusion limitation from differences that might result in the comparison of the activation energy (E_a) of both cases *(4,5)*.

The experimental test was conducted with 0.5 g (dry wt) of immobilized enzyme and the same procedure described above for the influence of IE mass on initial starch hydrolysis rate, whereas for the soluble enzyme, the same batch reactor was used with 0.5 mL of the stock enzyme, diluted 1 to 500 *(2)*. Two substrate concentrations were tested, namely 1 and 30% (w/v), and temperatures ranged from 35–70°C.

Initial Rate of Saccharification and Substrate Inhibition Test

This test was carried out also with the same procedure described above, but the immobilized enzyme mass was fixed at 0.57 g (dry wt), and starch concentration varied from 2–300 g/L (total of 24 determinations, with new enzyme for each case). Total reaction time decreased from 30 min used for more concentrated solutions down to 16 min for more diluted ones, so that the rate of hydrolysis in each case could be considered constant and equal to the initial rate of hydrolysis at that starch concentration.

Plotting the initial rate of hydrolysis vs starch concentration shows the influence of substrate inhibition. The same data treated as described in ref. *(2)*, Eq. (23), and the fitting description below it yield the Haldane parameters: K_{m4} (Michaelis-Menten constant for the susceptible oligosaccharides), V_{m4} (maximum velocity constant for the hydrolysis of the susceptible oligosaccharides), and K_S (substrate inhibition constant).

Fixed- and Fluidized-Bed Reactor Modeling

The multisubstrate model that was developed for batch saccharification of liquefied cassava starch with soluble amyloglucosidase *(2)* is here adapted for immobilized enzyme and continuous fixed- or fluidized-bed reactors. The main features of the model are preserved, and only minor modifications are necessary.

Earlier work *(6)* has shown that for the liquid fluidized beds of these systems, liquid axial dispersion is of intermediate level (dispersion number < 0.03), and therefore when used as a reactor, the performance of these fluidized beds will not deviate significantly from a plug flow reactor (PFR) *(7a)*. Fixed-bed reactors with the same range of variables should have even smaller dispersion. Consequently, both fixed- and fluidized-bed reactors with immobilized amyloglucosidase have been modeled as a PFR.

For constant density systems, as in the case of starch saccharification, both batch and PFR reactors lead to equivalent performance equations, in which the only difference is that where reaction time appears in the batch equation, residence time appears in the case of PFR *(7b)*. For this reason, the same computer program developed for saccharification of cassava starch in a batch reactor *(2)* has been used here with the fixed- and fluidized-bed reactors. To calculate the real residence time (t_R) in these reactors, the interstitial liquid volume (V_L) was considered the relevant volume.

$$t_R = V_L/v = (V_T\varepsilon)/v = M_e\varepsilon/[\rho_p(1-\varepsilon)v] \quad (1)$$

The parameters of the model related to maximum initial hydrolysis velocities now are given in units of mol/(h g_{IE}), and therefore the ratio of immobilized enzyme to liquid volume inside the reactor (E) is an important experimental datum. Interstitial liquid volume was also considered the relevant liquid volume to calculate this ratio. It is constant for fixed bed, since a fixed bed has a constant volume, but is different for each bed porosity (ε) in the fluidized bed, because this bed has higher expansions for greater flow rates.

$$E = M_e / V_L = M_e /(V_T\varepsilon) = \rho_p(1-\varepsilon)/\varepsilon \quad (2)$$

The apparent glucose inhibition parameter remains as the only adjustable parameter to make the model fit the reactor data.

Model Hypothesis

1. Since the substrate is starch that has been extensively hydrolyzed with α-amylase, the average degree of polymerization (n) of the remaining oligosaccharides with degree of polymerization > 3, was assumed to be 5, based on Reilly's description of α-amylase action *(8)*.
2. These oligosaccharides are considered to be formed of two fractions called G_4 and G_6. G_4 is more susceptible to hydrolysis, comprises approx 77% of the molecules, and contains the α-1,4 chemical bonds that are rapidly hydrolyzed. The second fraction, G_6, is resistant to hydrolysis, comprises the remaining 23% of the molecules, and contains the α-1,6 chemical bonds associated with branching that are hydrolyzed at a slower rate than the α-1,4 bonds *(9)*.
3. Saccharification proceeds through multiple reactions that occur simultaneously and are divided into three classes:
   a. Hydrolysis reactions of oligosaccharides with degree of polymerization (n) > 3 are lumped together as a single class that is divided in the two aforementioned fractions—susceptible (G_4) and resistant (G_6):

$$\text{Susceptible oligos. } (G_4) + (n-3)\,H_2O \rightarrow \text{maltotriose } (G_3) + (n-3)\,\text{glucose (G)} \quad (3)$$

$$\text{Resistant oligos. } (G_6) + (n-3)\,H_2O \rightarrow \text{maltotriose } (G_3) + (n-3)\,\text{glucose (G)} \quad (4)$$

   b. Hydrolysis of maltotriose (G_3) produces maltose (G_2) and is reversible:

$$\text{Maltotriose } (G_3) + H_2O \rightleftarrows \text{maltose } (G_2) + \text{glucose (G)} \quad (5)$$

   c. Hydrolysis of maltose produces glucose (G) and is reversible:

$$\text{Maltose } (G_2) + H_2O \rightleftarrows 2\ \text{glucose (G)} \quad (6)$$

4. Glucose can undergo condensation to isomaltose (G_I). This reaction is reversible:

$$2\ \text{glucose (G)} \rightleftarrows \text{isomaltose } (G_I) \quad (7)$$

5. There is product inhibition (K_i) in the case of glucose (G), for reactions given by Eqs. (3)–(6).
6. There is substrate inhibition (K_S) by oligosaccharides with degree of polymerization >3, but not for maltotriose and maltose.

7. The various substrates (G_4,G_6,G_3,G_2) compete for the amyloglucosidase active site.
8. During saccharification, water in the reaction medium is in excess, and therefore, its concentration is assumed constant.
9. Thermal deactivation of the enzyme was not considered in this work, since at 45°C and 30% substrate concentration, it was observed that amyloglucosidase stability is high *(3)*.
10. The reactors are modeled by the isothermal, plug flow, one-dimensional pseudohomogeneous model.

Hydrolysis Rate and Mass Balance Equations

Given the aforementioned considerations, Eqs. (8)–(21) apply:

1. Hydrolysis rate for the susceptible oligosaccharides (r_4):

$$r_4 = V_{m4}\mathrm{E}\,\mathrm{G}_4 / [K_{m4}(1 + \mathrm{G}/K_i) + \mathrm{G}_4 + \mathrm{G}_2K_{m4}/K_{m2} + \mathrm{G}_3K_{m4}/K_{m3} + \mathrm{G}_6K_{m4}/K_{m6} + \mathrm{G}_4^2/K_S] \quad (8)$$

2. Hydrolysis rate for the resistant oligosaccharides (r_6):

$$r_6 = V_{m6}\mathrm{E}\,\mathrm{G}_6 / [K_{m6}(1 + \mathrm{G}/K_i) + \mathrm{G}_6 + \mathrm{G}_2K_{m6}/K_{m2} + \mathrm{G}_3K_{m6}/K_{m3} + \mathrm{G}_4K_{m6}/K_{m4} + \mathrm{G}_6^2/K_S] \quad (9)$$

3. Rate of consumption of susceptible (G_4) and resistant (G_6) oligosaccharides:

$$\mathrm{dG}_4/\mathrm{dt}_\mathrm{R} = -r_4 \quad (10)$$

$$\mathrm{dG}_6/\mathrm{dt}_\mathrm{R} = -r_6 \quad (11)$$

4. Rate of hydrolysis of maltotriose (r_3):

$$r_3 = V_{m3}\,\mathrm{E}\,(\mathrm{G}_3 - \mathrm{G}_2\mathrm{G}/K_{eq3})/[K_{m3}(1 + \mathrm{G}/K_i) + \mathrm{G}_3 + \mathrm{G}_2K_{m3}/K_{m2} + \mathrm{G}_4K_{m3}/K_{m4} + \mathrm{G}_6K_{m3}/K_{m6}] \quad (12)$$

At equilibrium:

$$K_{eq3} = (\mathrm{G}_{2eq}\mathrm{G}_{eq})/\mathrm{G}_{3eq} \quad (13)$$

5. Rate of hydrolysis of maltose (r_2):

$$r_2 = V_{m2}\mathrm{E}(\mathrm{G}_2 - \mathrm{G}^2/K_{eq2})/[K_{m2}(1 + \mathrm{G}/K_i) + \mathrm{G}_2 + \mathrm{G}_3K_{m2}/K_{m3} + \mathrm{G}_4K_{m2}/K_{m4} + \mathrm{G}_6K_{m2}/K_{m6}] \quad (14)$$

At equilibrium:

$$K_{eq2} = \mathrm{G}_{eq}^2/\mathrm{G}_{2eq} \quad (15)$$

6. Net rate of formation of maltotriose and maltose:

$$dG_3/dt_R = r_4 + r_6 - r_3 \quad (16)$$

$$dG_2/dt_R = r_3 - r_2 \quad (17)$$

7. Rate of formation of isomaltose (r_I) resulting from the α-1,6 condensation of two molecules of glucose (Eq. [7]):

$$r_I = V_{IM}E(G^2 - G_I/K_{eqI}) \quad (18)$$

At equilibrium:

$$K_{eqI} = G_{Ieq}/G_{eq}^2 \quad (19)$$

$$dG_I/dt_R = r_I \quad (20)$$

8. Net rate of glucose formation:

$$dG/dt_R = (n - 3)(r_4 + r_6) + r_3 + 2(r_2 - r_I) \quad (21)$$

Conversion of the multiple substrates present in the liquefied starch solution (G_2, G_3, G_4, G_6) was calculated on a mass basis by the following equation:

$$X_A = 100f(C_g - C_{gi})/[C_{40} - f(C_{gi} - C_g)] \quad (22)$$

RESULTS AND DISCUSSION

Influence of Immobilized Enzyme Mass on Initial Starch Hydrolysis Rate

Figure 1 shows the initial starch hydrolysis rate as a function of the mass of immobilized enzyme used inside the basket. It can be observed that a linear relation holds up to 0.6 g of IE, equivalent to approx 50 mg/L of immobilized enzyme protein. Although starch concentration is relatively low (1% w/v) it is sufficient to ensure complete saturation of the enzyme's active site (200 mg of liquefied starch/mg of protein). These results allow one to select 0.57 g (dry weight) as the IE mass used to determine the initial rate of saccharification and substrate inhibition. Since starch concentration varied 2 to 300 g/L, an IE mass near to the upper limit is a good choice so that high initial velocities would be obtained also for the smaller starch concentrations.

Intraparticle Diffusion Limitations

Figure 2A show Arrhenius plots for the soluble and immobilized enzyme with 1% (w/v) starch concentration, whereas Fig. 2B is for 30% (w/v). The energy of activation (E_a) for each concentration is nearly coincident for both free and IE, and that indicates absence of intraparticle diffu-

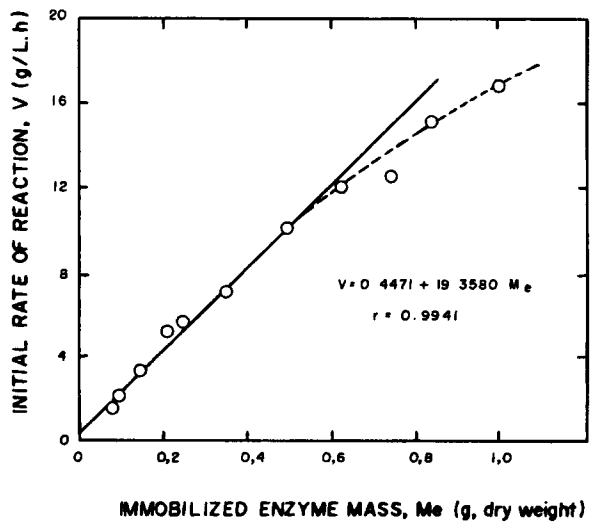

Fig. 1. Initial rate of saccharification of liquefied cassava starch by immobilized amyloglucosidase, 45°C, pH 4.5, 50 mL of substrate with starch concentration 1% (w/v), reaction time 16 min. Enzyme activity = 188.5 U/g IE.

sion limitation, but nevertheless, E_a is different for each concentration, namely about 9 and 13 kcal/mol respectively for 1 and 30% (w/v) starch. Since Arrhenius equation is only strictly valid for single elementar reactions *(10)*, the energy of activation derived from Arrhenius plot for complex kinetics may be a function of temperature and concentration. This has also been shown to be true for some reactions believed to be elementar *(11,12)*. The absence of intraparticle diffusion limitation is attributed to the large and regular pores of the CPS particle used to immobilize the enzyme and also to the slowness of the liquefied starch hydrolysis reaction at 45°C.

Initial Rate of Saccharification and Substrate Inhibition Test

Figure 3 is a plot of the initial rate of saccharification with IE vs concentration of the liquefied cassava starch. It shows clearly the presence of substrate inhibition for starch concentrations above 30 g/L. Maximal reaction velocity is observed around 50 g/L, whereas at 300 g/L (typical industrial starch concentration), the rate of saccharification is 23% lower. For the soluble enzyme *(2)*, maximum rate occurred at 90 g/L, and at 300 g/L, the rate was 25% lower. As mentioned in the methodology and described in ref. *(2)*, the Haldane parameters: Michaelis-Menten constant for the susceptible oligosaccharides (K_{m4}), maximum velocity constant for the hydrolysis of susceptible oligosaccharides (V_{m4}), and the substrate inhibition constant (K_S) were calculated from this data. Since the enzyme used in the reactors is 3.59 times more active than the enzyme used in this test, V_{m4} was corrected accordingly. The value of maximum rate of hydrolysis for resistant oligosaccharides (V_{m6}) was then taken as half V_{m4}. Maximum

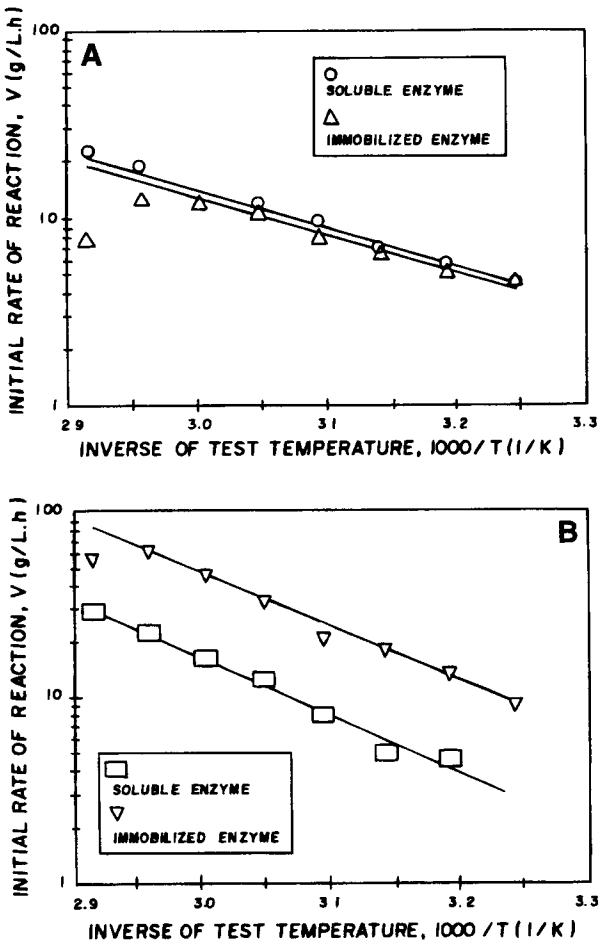

Fig. 2. Arrhenius plot for the saccharification reaction of liquefied cassava starch with soluble and immobilized amyloglucosidase. **(A)** Starch concentration, 1% (w/v); **(B)** starch concentration, 30% (w/v). For both cases, the pH was 4.5. Enzyme loading: soluble—0.5 mL of stock enzyme diluted 1 to 500; immobilized enzyme 0.5 g (dry wt).

velocities for maltose (V_{m2}) and isomaltose (V_{IM}) were taken from ref. *(2)* and expressed in terms of g_{IE} by multiplying them by the ratio of enzyme activities between the immobilized and soluble enzymes (676.8 U/g_{IE}/ 5358.4 U/mL). Maltotriose maximum velocity (V_{m3}) was then taken as twice the value of V_{m2}. The ratios between V_{m6} and V_{m4}, and V_{m3} and V_{m2} were taken from the literature *(9)*. Table 1 shows the complete set of parameters used to model the reactor data.

Fixed- and Fluidized-Bed Reactor Modeling

Figures 4 and 5 compare reactor performance data with the adapted multisubstrate model and show a very satisfactory agreement. The fluidized-bed reactor data (Fig. 5) are fitted with the same product inhibition

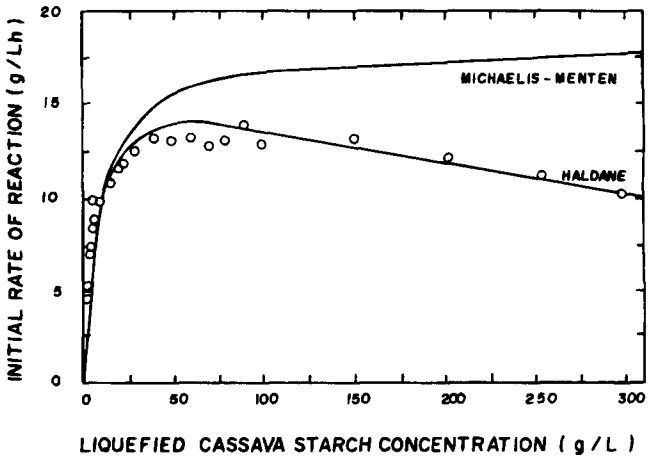

Fig. 3. Initial rate of liquefied cassava starch saccharification with immobilized amyloglucosidase, 45°C, pH 4.5. Reactor of basket type, 50-mL volume. Immobilized enzyme mass 0.57 g (dry wt); substrate concentration varied from 2–300 g/L.

Table 1
Complete Set of Kinetic Parameters Used with the Multisubstrate Model of Liquefied Cassava Starch Saccharification by Immobilized Amyloglucosidase

	Parameter	Parameter Values
Equilibrium constants	K_{eq2}	200 mol/L
	K_{eq1}	0.0544 mol/L
	K_{eq3}	8 mol/L
Michaelis-Menten constants	K_{m2}	0.0030 mol/L
	K_{m3}	0.004916 mol/L
	K_{m4}	0.004916 mol/L
	K_{m6}	0.004916 mol/L
Inhibition constants	K_S	0.2395 mol/L
	K_i (fixed bed)	0.030 mol/L
	K_i (fluidized bed)	0.200 mol/L
Maximum velocities	V_{m2}	0.009814 mol/h g_{IE}
	V_{m3}	0.019630 mol/h g_{IE}
	V_{m4}	0.01587 mol/h g_{IE}
	V_{m6}	0.007940 mol/h g_{IE}
	V_{IM}	0.00001378 L^2/mol g_{IE}

constant (K_i = 0.200 mol/L), whereas for the fixed-bed reactor data (Fig. 4), K_i is 0.030 mol/L. This result reveals a much stronger apparent inhibition in the fixed bed than in the fluidized bed.

Since inherent immobilized enzyme inhibition constants should be independent of the reactor type, diffusion limitations were investigated as

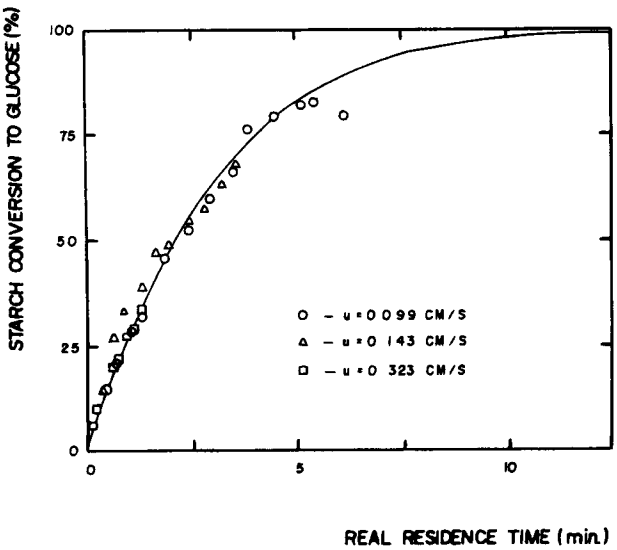

Fig. 4. Comparison of fixed-bed reactor data and reactor modeling for the saccharification of liquefied cassava starch with immobilized amyloglucosidase. Average bed porosity: 0.418. Starch concentration solution 30% (w/v), 45°C, pH 4.5. (u = superficial liquid velocity, ε = bed porosity)

possible causes for the difference in the apparent glucose inhibition values. However, previous work *(1)* has shown absence of interparticle diffusion limitation, and the data on Energy of Activation obtained in this work have indicated absence of intraparticle diffusion limitation. Therefore, the difference observed in apparent glucose inhibition with both types of reactors incorporates the effects of the macroscopic mixing differences of both reactors. Whereas in the fixed bed, particles are stationary and there is very little liquid axial mixing, in the fluidized bed, particles are free to move and engage in an overall slow recirculation pattern, leading to an axial liquid–solid mixing of intermediate value. For common reactions of positive order, macroscopic mixing in the reactors is detrimental to reactor performance, but for reactions with substrate inhibition, some axial mixing is beneficial because it lowers substrate concentration and, consequently, its inhibition. Channeling may also contribute to inferior performance of fixed bed IE reactors *(13)*.

CONCLUSIONS

The conclusions reached in this work are:

1. In the saccharification of liquefied cassava starch with immobilized amyloglucosidase, a strong substrate inhibition effect is observed for starch concentration above 30 g/L.

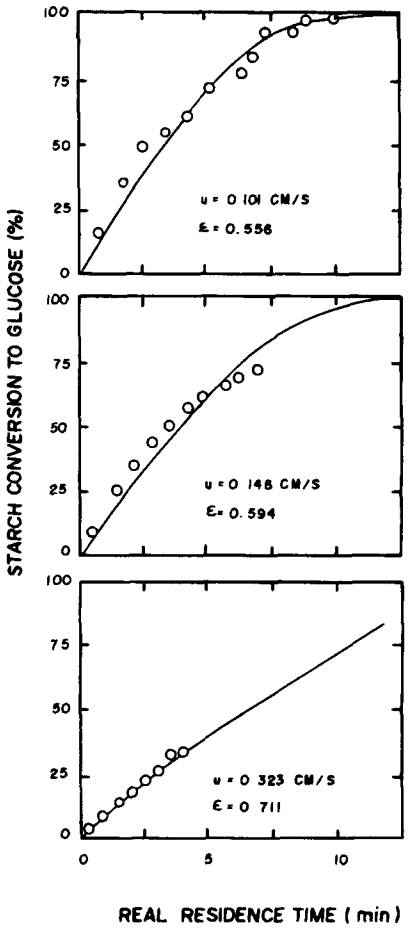

Fig. 5. Comparison of fluidized-bed reactor data and reactor modeling for the saccharification of liquefied cassava starch with immobilized amyloglucosidase. Starch concentration solution 30 % (w/v), 45°C, pH 4.5. (u = superficial liquid velocity, ε = bed porosity).

2. The multisubstrate starch hydrolysis model, previously developed for batch saccharification of liquefied cassava starch with soluble enzyme, was easily adapted for continuous fixed- and fluidized-bed reactors, giving very satisfactory agreement with experimental data.
3. Diffusional limitation, both inter- and intraparticle, was not observed for the reactor flow conditions used in this work and for the type of controlled pore silica of large pores used as support for enzyme immobilization.
4. The only parameter that had to be adjusted in the multisubstrate model to cope with experimental differences in fixed and fluidized bed results was apparent glucose inhibition.

5. For good modeling, fixed bed required a much lower apparent glucose inhibition constant than fluidized bed, and that means a much higher apparent glucose inhibition (about seven times). The difference should not result from differences in the intrinsic kinetic parameter in each bed, but would more likely be a consequence of the different macroscopic liquid mixing of each reactor type.

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NOMENCLATURE

C_{A0} initial starch concentration, 300 g/L
C_g glucose concentration, g/L
C_{ga} concentration of added glucose, g/L
C_{gi} glucose concentration at the start of saccharification, g/L
E mass of immobilized enzyme per volume of interstitial liquid, g_{IE}/L
f ratio of molecular weights for the anhydroglucose unit in starch and glucose, f = 162/180 = 0.9
G glucose molar concentration, mol/L
G_2 maltose molar concentration, mol/L
G_3 maltotriose molar concentration, mol/L
G_4 susceptible oligosaccharides molar concentration, mol/L
G_6 resistant oligosaccharides molar concentration, mol/L
G_I isomaltose molar concentration, mol/L
G_{eq} G_{2eq}, G_{3eq}, G_{Ieq} equilibrium molar concentration for glucose, maltose, maltotriose, and isomaltose, respectively, mol/L
K_{eq2} K_{eq3}, K_{eqI}, equilibrium constants for maltose (mol/L), maltotriose (mol/L), and isomaltose (L/mol), respectively
K_i apparent glucose inhibition constant, mol/L
K_{m2} K_{m3}, K_{m4}, K_{m6}, Michaelis-Menten constants for maltose, maltotriose, susceptible oligosaccharides, and resistant oligosaccharides, respectively, mol/L
K_S substrate inhibition constant, mol/L
M_e immobilized enzyme mass, dry wt, g
n average degree of polymerization, dimensionless
r_2 r_3, r_4, r_6, r_I, rate of reaction for maltose, maltotriose, susceptible oligosaccharides, resistant oligosaccharides, and isomaltose, respectively, mol/(L h)

t_R fluid real residence time, h
v volumetric liquid flow rate, cm^3/s
V initial rate of glucose production, g/(L h)
V_L interstitial liquid volume, $V_L = V_T (1 - \varepsilon)$, cm^3
V_{IM} second-order rate constant for isomaltose, L_2 (mol·h·mL of enzyme)
V_{m2} V_{m3}, V_{m4}, V_{m6}, maximum velocity constants associated with the reaction rate of maltose, maltotriose, susceptible oligosaccharides, and resistant oligosaccharides, respectively, mol/(h gIE)
V_T total reactor bed volume, $V_T = M_e/ [\rho_p(1 - \varepsilon)]$, cm^3
X_A conversion of liquefied starch to glucose, %
ε bed porosity
ρ_p particle density, $\rho_p = 0.939$ g/cm^3

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