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Glucoamylase Immobilization on a Magnetic Microparticle for the Continuous Hydrolysis of Maltodextrin in a Fluidized Bed Reactor

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ABSTRACT

Glucoamylase (GA) has been successfully immobilized through its carbohydrates previously oxidized with periodate onto a low-cost magnetic microparticle made of polyethyleneimine-coated magnetite crosslinked with glutaraldehyde (M-GAD) and derivatized with adipic dihydrazide (ADH). A stabilization posttreatment consisting of crosslinking its carbohydrates with ADH, increased the remaining activity from 54 to 71%, calculated on the V_m values and measured at 50°C and pH 4.5 with maltodextrin (DE 11–14) as substrate. This treatment also improved the enzyme stability and lowered the deactivation rate constant k_d to a third of its value.

A 30% maltodextrin solution has been continuously hydrolyzed at 50°C and pH 4.5 in a recycled, fluidized bed reactor (FBR) containing GA immobilized on these magnetic microparticles. They easily settled in this highly viscous medium because of their high density (5 g/mL), and washout of ultrafines was prevented by surrounding the top of the bed with an electromagnet. The small particle size (20 μm) allowed a high enzyme loading in the reactor and also a high bed voidage, which is recommended to avoid extensive pressure drop and consequent channeling problems. The kinetic of hydrolysis fitted with

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the plug-flow model; this is explained by the insignificant backmixing effects observed. After 2 wk of hydrolysis under process conditions leading to a conversion of 70%, which corresponds to a high-conversion syrup, the immobilized GA only lost 4% of its initial activity.

Index Entries: Magnetite; glucoamylase; glycoenzymes; oxidation; crosslinking; immobilization; maltodextrin hydrolysis; fluidized bed reactor; biosensor.

NOMENCLATURE

ADH	Adipic dihydrazide
D_c	Effective longitudinal dispersion coefficient based on interstitial velocity (cm^2/min)
d_p	Particle mean diameter (cm)
F	Flow rate (mL/min)
FBR	Fluidized bed reactor
g	Gravitational constant (cm/min^2)
GA	Glucoamylase
GA_{ox}	Oxidized glucoamylase
GAD	Glutaraldehyde
h_b	Bed height (cm)
K_m	Michaelis-Menten constant (g/mL or mM)
L	Enzyme loading on the particle (mg_E/g_M)
M	Polyethyleneimine coated magnetite
M-GAD	M crosslinked with glutaraldehyde
M-ADH	M-GAH derivatized with adipic dihydrazide
n	Constant of Richardson-Zaki
Pe	Peclet Number
Re	Reynolds Number
Re_{mf}	Reynods Number at minimal fluidization velocity
S_0	Initial substrate concentration (g/mL)
U	Unit (μmol/min)
u	Fluid velocity (cm/min)
u_{mf}	Minimal fluidization velocity (cm/min)
u_t	Maximal fluidization velocity (cm/min)
v_b	Bed volume (mL)
v_l	Liquid volume of the bed (mL)
v_p	Particle volume (mL)
V	Specific enzyme activity (U/mg_E)
V'	Enzyme activity reported to the particle wt (U/g_M)
V_m	Maximal enzyme activity (g/min·mL or U/mL) or maximal specific enzyme activity (U/mg_E)
X	Substrate conversion (– or %)
α	Recycling coefficient
ϵ	Bed voidage
ν	Kinematic viscosity (cm^2/min)
ρ	Fluid density (g/mL)
ρ_p	Particle density (g_M/mL)

INTRODUCTION

The hydrolysis of maltodextrin with GA is an important process in the sugar industry for the production of high-conversion (DE 60–70) and high-dextrose (DE 95) syrups (*1,2,3*). In the 1970s, the immobilization of GA was studied extensively, since processes using insoluble enzymes can work continuously. This lowers the operating cost at a higher enzyme loading, which reduces the reactor size, the reaction time, and consequently, the capital cost. Moreover, the biocatalyst normally lost and heat-activated in processes working with soluble enzymes can be recovered after use, which lowers the costs of materials and utilities.

However, at the end of the 1970s, Norman, having tried to use immobilized GA in a packed bed reactor (PBR), concluded that this technique suffered serious process and economical limitations (*2*). Since GA is produced at a relatively low cost by fermentation, the cost of the carrier and the immobilization procedure and the loss of initial enzyme activity after immobilization do not make this process economically more advantageous than that of working with soluble enzymes. Moreover, in a PBR, the substrate must be made free of particulate matters to prevent blocking of the bed, and on a large scale, the pressure drop is so high that it may provoke bed compaction and biocatalyst damage. The carrier used in their study was also highly porous, which increased the reversion phenomenon due to diffusional resistance.

Cabral et al. (1986) found better performances with the FBR in terms of mass transfer, pressure drop, substrate conversion, and enzyme stability; but the immobilization procedure was still expensive and gave a relatively low remaining activity (*4*). They concluded that a process working with immobilized GA could be economically viable only if a cheaper immobilization method was devised (*3*).

Recently, Freire et al. (1990) tried to achieve this goal by using, in a similar reactor, a low-cost chitin particle of 250 μm in mean diameter for the hydrolysis of a 15% prehydrolyzed manioc starch (*5*). Bon et al. had previously found a good remaining activity of 44% for GA immobilized on this support, calculated at pH 4.4 and 30°C with 1% maltose as substrate (*6*). Although they observed a high enzyme stability in the reactor with only 6% loss in activity after 20 d, this particle, due to its low density, may probably be washed out in an FBR containing a 30% maltodextrin solution, a concentration commonly used in the industry (*2,3,4*).

This challenge has been taken up in our previous work (*7*), which consisted of immobilizing GA on a low-cost and high-density magnetic microparticle made of polyethyleneimine-coated magnetite crosslinked with glutaraldehyde (M-GAD) and tailored by Dekker in 1989 for the immobilization of β-glucosidase (*8–9*). A remaining activity of 43% comparable to that found by Bon et al. and calculated on the V_m values at similar con ditions, i.e., pH 5 and 30°C with maltose as substrate, has been found by direct coupling of GA on this particle (*6*). This percentage was

drastically improved to 88% by derivatizing the particle with adipic dihydrazide as spacer (M-ADH) and by immobilizing GA through its carbohydrates previously oxidized with periodate. With maltodextrin as substrate, the remaining activity decreased to 70%.

The use of magnetic particles in bioprocesses has been recently reviewed, and their principal advantage is that they can be easily separated by a magnetic field (*10–13*). This property facilitates their manufacture, the immobilization procedure, their handling in the reactor, and their recovery from the process liquor, thereby reducing capital and operating costs. The M-ADH particle is extremely easy to manufacture and only requires cheap reagents. Its micronic size of 20 μm in mean diameter and its irregular surface allow a high enzyme loading in the reactor; and its nonporous structure, coated with spacers, reduces the diffusional resistance problems.

Since the particle is economically attractive and easily settles in a 30% maltodextrin solution, the purpose of this work was to study the kinetic of the maltodextrin hydrolysis in an FBR and the stability of the system over a period of 2 wk. A similar process has already been studied by Gellf and Boudrant (1974) for the hydrolysis of N-benzoyl-L-arginine ethyl ester with papain immobilized by direct coupling on albumin-coated magnetite, a polymer far too expensive, nevertheless, to be used in industrial processes (*14*). Another advantage of magnetic particles in FBR is that particle washout can be prevented by simply surrounding the top of the column with an electromagnet.

The stabilization of immobilized GA has also been attempted in the present work by crosslinking its carbohydrate moities with ADH, a treatment proposed by Kozulic et al. for the stabilization of glycoenzymes (*15*).

THEORY

Plug-Flow Reactor Model

The plug–flow behavior of an FBR can be predicted by the following equation (*16*):

$$F X S_0 = K_m F \ln(1 - X) + V_m v_l, \quad (1)$$

where F is the volumetric flow rate (mL/min),
X is the substrate conversion,
S_0 is the initial substrate concentration (g/mL),
v_l is the liquid volume of the bed (mL),
K_m is the Michaelis constant (g/mL), and
V_m is the maximal enzyme activity (g/min·mL).

The substrate conversion X is the molar ratio of the glucose concentration measured in the overflow to the initial equivalent glucose concentration in maltodextrin, which was assumed to have a mol wt of 162 g/mol. The liquid volume of the bed is $v_l = v_b - v_p = (1 - v_p/v_b)\ v_b = \epsilon\ v_b$, where v_b is the bed volume (mL), v_p is The particle volume in the bed (mL), and ϵ is the bed voidage.

Determination of the Mean Diameter of the Particle

At low Reynolds number, the mean diameter of the particle d_p (cm) can be deduced from the Stokes law (*17*):

$$d_p^2 = u_t \nu / (\rho_p/\rho - 1)\, g, \tag{2}$$

where u_t is the maximal fluidization rate (cm/min),
ρ_p is the particle density (g/mL),
ρ is the fluid density (g/mL),
ν is the kinematic viscosity (cm²/min), and
g is the gravitational constant = 9.81 m/s² = 353.16 × 10⁴ cm/min².

The maximal fluidization velocity u_t (cm/min) was estimated by measuring the drop velocity of the particle bed front in the FBR after turning off the substrate flow.

Determination of Maximal and Minimal Fluidization Velocity

The minimal fluidization velocity u_{mf} (cm/min) was determined according to two correlations (*17*):

$$u/u_t = \epsilon^n \tag{3}$$

—called the Richardson-Zaki law, where n is the constant of Richardson-Zaki. In a log-log graph, the bed voidage decreases linearly with the fluid velocity u (cm/min) until a break point corresponding to the minimal fluidization velocity, where it reaches its minimal volume.

$$u_{mf} = d_p^2 (\rho_p/\rho - 1)\, g / 1650\, \nu^2 \tag{4}$$

This correlation is valid if $Re_{mf} < 20$, where Re is the Reynolds number:

$$Re = u d_p/\nu \tag{5}$$

Correlations for Measuring the Magnitude of the Dispersion Effects

The magnitude of the backmixing effects may be estimated by the dispersion number (*16*):

$$D_c / uh_b = d_p / Peh_b \quad (6)$$

where D_c is the effective longitudinal dispersion coefficient based on interstitial velocity (cm²/min). h_b is the bed height (cm), and Pe is the Peclet Number. The Peclet Number can be correlated to the Reynolds Number by the Chung and Wen equation claimed to cover $10^{-3} < Re < 10^3$ and $0.4 < \epsilon < 0.8$:

$$Pe/Z = 0.20 + 0.011\, Re^{0.48} \quad (7)$$

and for the fluidized beds,

$$Z = Re_{mf}/Re = u_{mf}/u \quad (8)$$

MATERIALS AND METHODS

Chemicals

GA E.C.3.2.1.3 (6 *U*/mg at 25°C), glycogen as substrate- and Glucose Oxidase (GOX) E.C.1.1.3.4 (250 *U*/mg at 25°C), and glucose as substrate (grade I) from Aspergillus niger were purchased from Boehringer. D(+)-glucose was provided by Prolabo, GAD and sodium periodate by Merck, magnetite by Alfa Products, polyethyleneimine (PEI) M.W. 30,000–40,000 by Serva, and ADH by Aldrich. The protein test was purchased from Bio-Rad. Maltodextrin GLUCIDEX 12 D (DE 11–14; composition: glucose 1%, maltose 2%, polysaccharides 97%) was a gift from Roquette Frères S.A. (France).

GA Oxidation

GA was oxidized with periodate in 50 m*M* acetate buffer at pH 5 as described previously (*7*). In a 5 mL test tube, 120 μL of a 50 m*M* periodate solution was added to 1 mL of 1 mg/mL enzyme solution. The solution was adjusted to 1.5 mL with the same buffer and reacted on a rollermixer for 6 h at 4°C in the dark. Periodate was removed by passing the solution through PD-10 Sephadex columns (Pharmacia). Dialysis was preferred for larger enzyme quantities, as this required the reactor. The protein concentration in the final solution was determined by the Bio-Rad test. The oxidized GA will be referred to as GA_{ox}.

GA Immobilization

GA_{ox} was immobilized on M-GAD and derivatized with M-ADH as described previously (*7*). Enzyme immobilization has always been performed at a loading L below the maximal loading capacity of the particle, i.e., 26 mg_E/g_M for GA_{ox}, since in this condition no enzyme concentration was found in the supernatants because of the high reactivity of the enzyme

surface (*7*). For the preliminary kinetic tests, a 5 mL test tube containing 5 mg of M-ADH and 68 μg of GA_{ox} in 1 mL of a 50 m*M* acetate buffer pH 4.5 was agitated for 2 h at 4°C on a roller mixer ($L = 13.6$ mg_E/g_M). After removal of the supernatant, the particles were washed twice at 4°C with 1 mL KCl 1 *M* in 0.2 *M* acetate buffer pH 4.5, for 1 h and for 20 min, respectively. For the reactor, 105 mg of GA_{ox} were immobilized onto 5 g of M-ADH ($L = 21$ mg_E/g_M) in 500 mL of a 50 m*M* acetate buffer. Immobilized GA_{ox} will be referred to as M-ADH-GA_{ox}.

Carbohydrates Crosslinking of Immobilized GA

M-ADH-GA_{ox} was crosslinked according to the method of Kozulic (*15*). Five mL test tubes containing 2 mL of 50 m*M* acetate buffer pH 4.5 and 5 mg of M-ADH-GA_{ox} were gently agitated at 4°C on a roller mixer. ADH was added at different concentrations varying from 2 to 12.5 m*M*. The crosslinking reaction was stopped after 1 h by magnetic removal of the particles. For the reactor, 5 g of M-ADH-GA_{ox} were treated for 1 h in 500 mL of a 50 m*M* acetate buffer containing 4 m*M* ADH.

Measurement of Glucose Concentration and GA Activity with a Glucose Biosensor

The biosensor system consists of a Gluc 1 electrode connected to a polarograph type PRGE manufactured by Solea-Tacussel (France), and the anodic current was recorded by a Sefram-Servotrace. The activity test is based on the detection of H_2O_2 produced by glucose hydrolysis with GOX immobilized on a preactivated polyamide membrane Pall-Immunodyne® of 0.45 μm in pore diameter tightly pressed on the platinum tip of the transducer with a screw cap. For this purpose, the potential of the platinum anode was fixed at +650 m*V* vs an Ag/AgCl reference electrode (*18,19*).

The tests were performed in a small thermostated reactor always containing 10 mL of a 0.1 *M* acetate buffer pH 4.5, 0.1 *M* KCl. For determination of the glucose concentration present in a sample or produced during the maltodextrin hydrolysis with GA, the sensor was previously calibrated with glucose solutions of defined concentrations. The reaction medium was stirred magnetically, except for immobilized enzymes, which required an overhead agitator.

The biosensor system measures the enzyme activity in terms of U/mL of reaction medium ($U/mL = \mu mol_{glucose}/min \cdot mL$). For soluble enzymes, the division of this activity by the protein concentration in the medium gives the catalysis velocity *V* or the specific enzyme activity in U/mg protein (U/mg_E). For immobilized enzymes, its division by the particle concentration gives the enyzme activity reported to the particle weight *V'* (U/g_M), and their specific activity *V* (U/mg_E) is calculated by dividing *V'* by the loading *L* of the enzyme on the particle (mg_E/g_M).

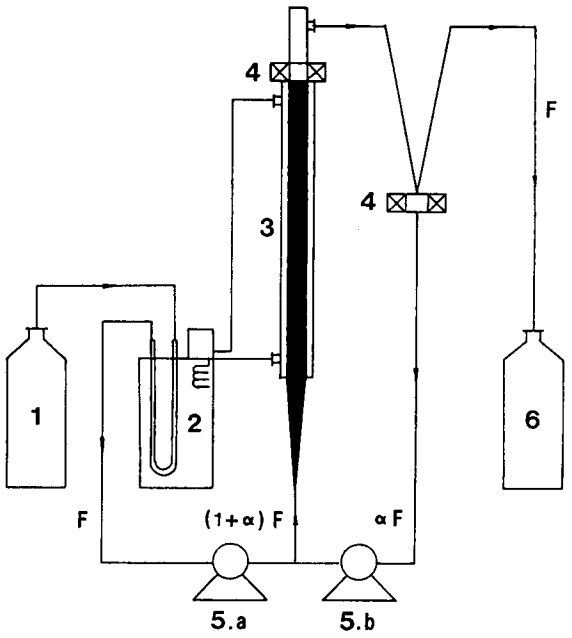

Fig. 1. Flow diagram of the pilot-plant reactor: (*1*) feeding tank stored at 4°C in a fridge; (2) water bath thermostated at 50°C with a Thermomix; (*3*) jacketed column; (*4*) electromagnets; (*5*) Pharmacia (a) and Watson-Marlow (*b*) peristaltic pumps; and (*6*) storage tank.

Since GA fits with the Michaelis-Menten kinetic, the kinetic constants K_m (m*M*) and V_m (U/mg$_E$) were calculated from the Lineweaver-Burk plot. For the immobilized enzymes, they always refer to the apparent constants. The remaining activity after oxidation and immobilization was referred to respectively as the ratio of the V_m of the oxidized and immobilized enzyme to that of the native enzyme.

Reactor System

The flow diagram of the pilot-plant reactor is illustrated in Fig. 1. A 30% maltodextrin solution in 100 m*M* acetate buffer pH 4.5 stored at 4°C to avoid contamination was preheated in a glass vessel immersed in a water bath thermostated at 50°C with a Thermomix 1420 (Braun) before passing through the FBR. The reactor consists of a glass column of 1.4 cm inside diameter and 65 cm length, conical part included, thermostated at 50°C and containing 5 g of crosslinked M-ADH-GA$_{ox}$ expanded to a maximal volume of 70 mL corresponding to a bed height of 55 cm. For this purpose and for the overflow recycling, a Pharmacia P1 and a Watson-Marlow 101U peristaltic pump were used respectively. The top of the bed was surrounded by an electromagnet producing a magnetic inductance intensity of 4 mT to avoid particule washout. Ultrafines that could pass

Table 1
Kinetic Constants of Native, Oxidized, and Immobilized Glucoamylase at 50°C, pH 4.5 with Maltodextrin as Substrate

	K_m (m*M*)	V_m (*U*/mg$_E$)
GA native	0.44	78
GA oxidized	0.60	89
M-ADH-GA$_{ox}$	1.68	42

through the magnetic field were fully recycled, since another electromagnet placed at the bottom of the junction between the recycling and the outflow forced them to fall down.

RESULTS AND DISCUSSION

Kinetic of Native, Oxidized, and Immobilized GA

In our previous work, we have found that the remaining activity of GA$_{ox}$—determined at 30°C, pH 5 with maltose as substrate in relation to the periodate concentration used—followed a gaussian curve. At the optimal periodate concentratiin (120 μL of a 50 m*M* periodate solution in 1 mL of a 1 mg/mL GA solution), the V_m of the GA$_{ox}$ was 7% higher than that of the native enzyme. Marek et al., having observed a similar phenomenon with GOX and invertase, have explained that this increase in activity was probably caused by partial destruction of the carbohydrate moieties that facilitate the accessibility of the substrate to the active site (*20,21*). The decrease in activity observed at higher periodate concentrations may be due to the oxidation of some amino acids normally masked by the carbohydrates or to a too-high breakage of carbohydrate residues, which normally stabilize glycoenzymes (*22,23*). We have also observed that at its highest V_m, the oxidized enzyme exhibited its highest K_m, which means that the faster the substrate accessibility to the active site, the lower its affinity for the substrate (*7*).

The remaining activity after GA$_{ox}$ immobilization on M-ADH was as high as 70% with maltodextrin as substrate and did not depend on the enzyme loading, as observed for GOX. Table 1 reports the kinetic constants of native, oxidized, and immobilized GA measured at 50°C and pH 4.5, operational conditions commonly used for the maltodextrin hydrolysis in reactors working with immobilized GA (*4*). The remaining activities after oxidation and immobilization were 114 and 54%, respectively. By comparing these results with those previously obtained at 30°C, it may be

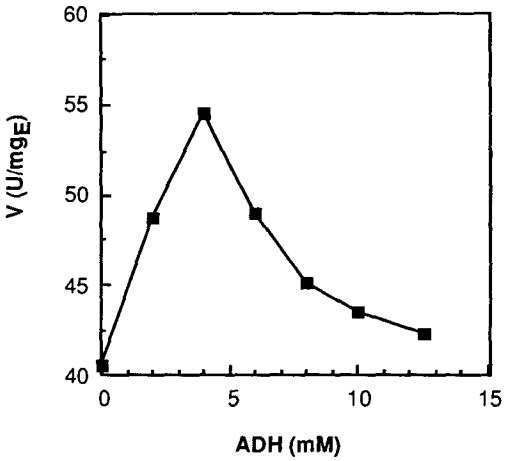

Fig. 2. Activity of immobilized GA_{ox} in relation to the ADH concentration present in the solution used for the crosslinking of its carbohydrates. Activity tests were performed at 50°C and at 2.25 g/L maltodextrin in 0.1 *M* acetate buffer pH 4.5, KCl 0.1 *M*.

concluded that the remaining activity after oxidation is quite similar, which means that this treatment does not heat-deactivate GA and that the remaining activity after immobilization decreases when the temperature increases. This also means that the immobilization does not heat-stabilize GA (*7*).

Effect of Carbohydrate Crosslinking on the Kinetic and Stability of Immobilized GA

Kozulic et al. (1987) have found that the crosslinking of soluble glycoenzymes oxidized with periodate and crosslinked with ADH increased their stability (*15*). A preliminary kinetic study on the crosslinking of oxidized carbohydrates still present on the surface of M-ADH-GA_{ox} was attempted in our previous work (*7*). At a final concentration of 12.5 m*M* of ADH in the treatment solution, we have found that both the V_m and the K_m of M-ADH-GA_{ox} increased. In the present work, the optimization of the ADH concentration and the possibility of enzyme stabilization by this treatment have been studied. The activity tests were always performed at 2.25 g/L maltodextrin.

The ADH concentration required for carbohydrate crosslinking is critical. A too-high concentration may prevent the crosslinking, since only one reaction of the two hydrazide groups of ADH with an oxidized carbohydrate may occur. A too-low concentration may not complete the crosslinking of the whole oxidized carbohydrates. Figure 2 shows the influence of the ADH concentration on the activity of M-ADH-GA_{ox}. An increase of 35% in activity may be observed at 4 m*M* ADH in the treatment solution.

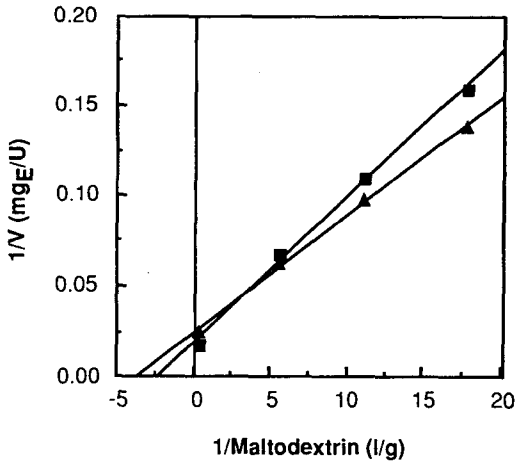

Fig. 3. Lineweaver-Burk plots of immobilized GA_{ox} before (▲) and after (■) crosslinking of its carbohydrates in a 4 m*M* ADH solution. Experimental conditions were as indicated in Fig. 2.

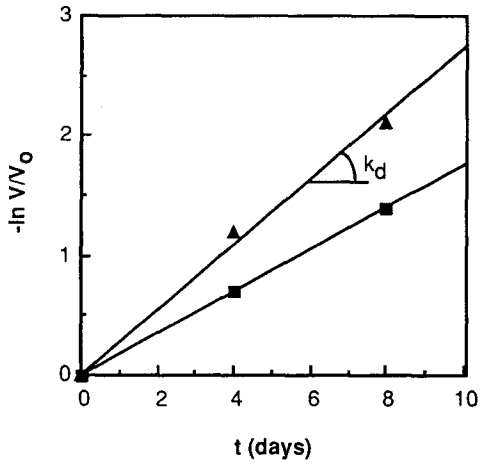

Fig. 4. Determination of the deactivation rate constant k_d (day^{-1}) of uncrosslinked (▲) and crosslinked (■) immobilized GA_{ox} stored at 50°C in a 50 m*M* acetate buffer, pH 4.5. Experimental conditions were as indicated in Fig. 2.

Figure 3 represents the Lineweaver-Burk plots of M-ADH-GA_{ox} before and after crosslinking with 4 m*M* ADH. The K_m value increases from 1.68 to 2.74 m*M* and the V_m value from 42 to 55 U/mg_E, which means an increase of the remaining activity from 54 to 71%.

The stability of M-ADH-GA_{ox} before and after crosslinking with 4 m*M* ADH have also been compared during 8 d at 50°C in a 50 m*M* buffer, a molarity low enough to reduce the stabilizing effect of salt (*23*). A significative stabilization effect has been observed and quantified by the deactivation constant k_d (day^{-1}) calculated from a first-order decay rate model $-ln(V/V_0)=k_dt$, where V_0 is the activity time $t=0$ (*4,15*). From Fig. 4, k_d

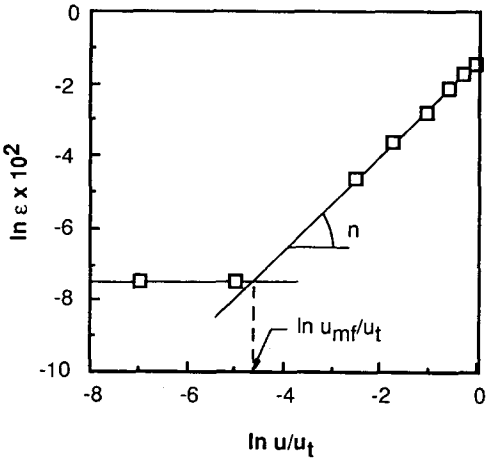

Fig. 5. Determination of the minimal fluidization velocity according to the log-log representation of the Richardson-Zaki law.

values of 0.262 day^{-1} and 0.175 day^{-1} were found respectively for M-ADH-GA_{ox} before and after crosslinking, which means a decrease of a third of the deactivation constant.

Maltodextrin Hydrolysis in an FBR

Determination of the FBR Parameters

The minimal and maximal fluidization velocity, the mean diameter and density of the particle, the viscosity of the fluid, and the porosity of the bed are important parameters to determine in order to study an FBR. These parameters were measured at 50°C and in a 30% glucose solution to avoid any change in fluid viscosity. The physiochemical properties of this solution are reported in the literature, and they were assumed to be similar to those of a highly hydrolyzed 30% maltodextrin solution.

Values for the fluid density ρ and the kinematic viscosity ν have been found to be 1.125 g/mL (*24*) and 0.633 cm^2/min (Roquette communication), respectively. The particle density ρ_p is 5 g/mL, and therefore, since the reactor contains 5 g of M-ADH-GA_{ox}, the particle volume v_p is 1 mL (*7*). A particle mean diameter d_p of 2×10^{-3} cm (20 μm) was obtained from Equation 2.

The maximal fluidization velocity u_t was estimated by measuring the drop velocity of the particle bed front in the FBR after turning off the substrate flow, which was 4.4 cm/min. The minimal fluidization velocity, u_{mf} was at first approximated by plotting the Equation 3 of Richardson-Zaki in a log-log diagram (Fig. 5), which gave a value of 0.038 cm/min. The minimal bed volume and the corresponding bed voidage ϵ_{mf} were found to be 14 mL and 0.928, respectively, and the Richardson-Zaki constant equals 1.27. Although $\epsilon_{mf} < 0.4$, Equation 4 gave a value of 0.048 cm/min for u_{mf}, which is a good approximation of this found experimentally.

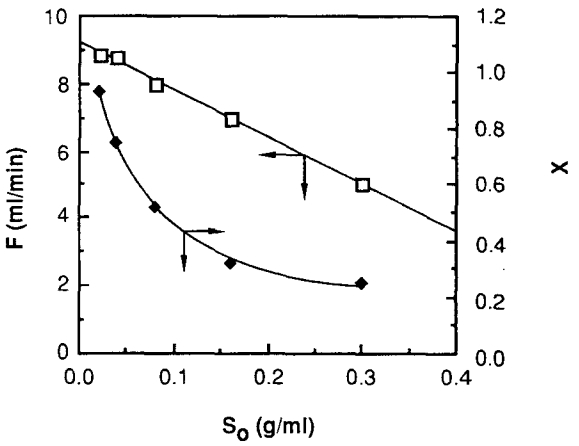

Fig. 6. Influence of the initial maltodextrin concentration on the flow rate required to obtain a fluidized bed of 70 mL and on the corresponding conversion obtained after one pass in the reactor. The maltodextrin solution was buffered at pH 4.5 with a 0.1*M* acetate buffer, and the reactor was thermostated at 50°C.

Determination of the Magnitude of the Backmixing Effects

Backmixing effects may significantly reduce reactor productivity, and its magnitude may be estimated by the dispersion number D_c/uh_b. Equation 6 correlates it with the *Pe*, which requires Equations 5, 7, and 8 for its calculation. For a fluid velocity u of 4.3 cm/min corresponding to a bed volume of 70 mL, $Re=1.355\times10^{-3}$, $Z=0.009$, and $Pe=0.0018$. As the bed height equals 55 cm, $D_c/uh_b=0.02$. The Dispersion Number has a low value, which means that the backmixing effects will not be significant in the FBR.

Channeling across the bed, another drawback that may occur in FBR and that results from a too-high pressure drop across the bed, has not been observed. Since the higher the bed voidage the lower the pressure drop, this may be explained by the high value of the bed voidage, which was as high as 0.986 in this case (*17*).

Kinetic Studies

Because backmixing effect did not seem significant, kinetic studies in a nonrecycled FBR at a constant bed volume v_b of 70 mL and at different substrate concentrations have been attempted in order to see if the conversion fitted with the plug-flow model. The bed voidage ϵ is 0.986, and the liquid volume of the bed v_l is 69 mL. Figure 6 shows the evolution of the flow rate in relation to the substrate concentration and the corresponding conversion. These values were plotted in a diagram according to Equation 1 (Fig. 7). The linear relationship shows that the reactor kinetic fits with the plug-flow model, and the kinetic constants can be deduced from the corresponding equation:

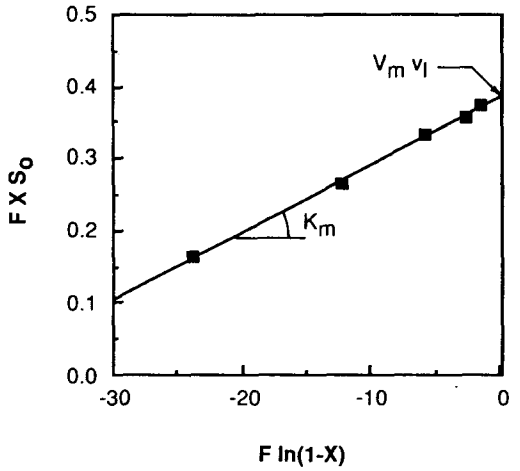

Fig. 7. Determination of the kinetic constants of immobilized GA in the fluidized bed reactor according to the plug-flow model. The experimental values of the parameters come from Fig. 6.

$$FX\,S_0 = 0.009\,F\ln(1-X) + 0.382$$

where $K_m = 0.009$ g/mL = 50 mM and
$V_m = 0.382/v_l = 0.0055$ g/mL·min = 30.6 U/mL

As the enzyme loading in the reactor is 1.5 mg_E/mL, $V_m = 20.4\,U/mg_E$.

The decrease in V_m compared to that measured in the batch reactor was foreseeable, since particles are not as highly agitated and dispersed in the FBR, where particles tended to flocculate. This phenomenon is a combined effect of the presence of sugar polymers and electrolytes, and of the particles surface attraction due to their protein coating. It may explain the drastic increase in the K_m value, which means a decrease in substrate accessibility to the active site. It may be noticed from Fig. 6 that the flow rate required for the fluidization of the bed to a volume of 70 mL varies with the substrate concentration according to a linear relationship: $F = 9.2 - 14\,S_0$.

Maltodextrin Hydrolysis in a Recycled FBR

High conversion of a 30% maltodextrin solution can only be obtained if the overflow is partially recycled. Figure 8 shows the evolution of the conversion in relation to the initial flow rate F and the recycled flow rate αF required for the fluidization of the bed to a constant volume of 70 mL. α is referred to as the recycling coefficient. The flow rate across the column $(1+\alpha)F$ is not constant, however, since the lower the initial flow rate F, the higher the conversion and therefore, the lower the viscosity.

It may be deduced from Fig. 9 that the reactor can produce a high-conversion syrup (DE 60–70) at an initial flow rate ranging from 0.75 to 1.5 mL/min, corresponding to a residence time from 90 to 45 min. However, the production of a high-dextrose syrup (DE 95) should have required a

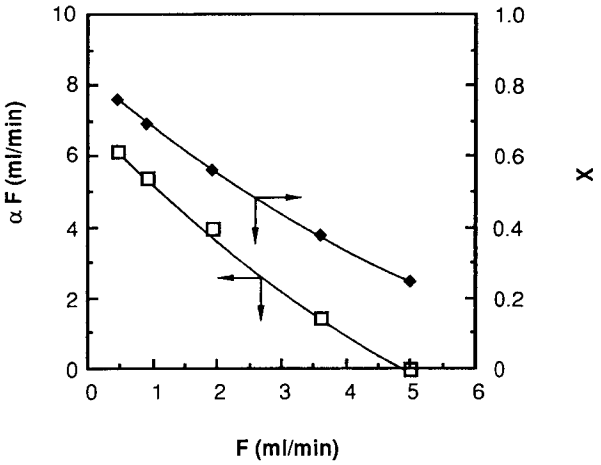

Fig. 8. Influence of the flow rate F of a 30% maltodextrin solution passing through the recycled fluidized bed on the recycled flow rate αF necessary to fluidize the bed to a volume of 70 mL and on the corresponding conversion. Operational conditions were as indicated in Fig. 6.

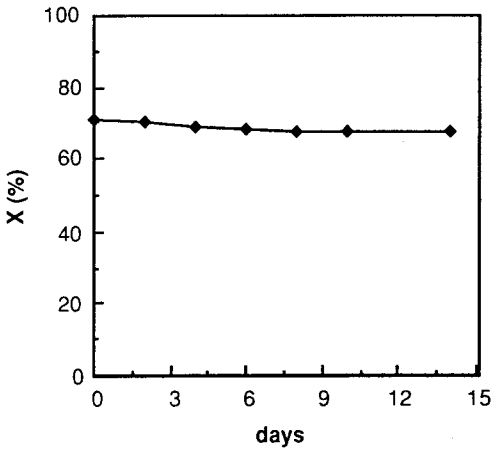

Fig. 9. Evolution of the conversion of a 30% maltodextrin solution during a 2-wk period, passing through the recycled fluidized bed reactor at a flow rate F of 0.75 mL/min. Operational conditions were as indicated in Fig. 6.

too-low initial flow rate. A higher bed containing more immobilized enzymes would have been necessary, but it was out of the scope of this study.

Stability Study

A 30% maltodextrin solution was continuously hydrolyzed in the recycled FBR during 2 wk at an initial flow rate of 0.75 mL/min, giving an initial conversion of 71%. Figure 9 shows the evolution of the conversion, which lost only 4% after 2 wk. Although the test period was not long

enough to predict the half-life of the immobilized GA, it would have been certainly longer than that of 32 d found by Cabral et al. under similar operational conditions (*4*). With GA immobilized on chitin, Freire (1990) observed a loss in activity of only 6% after 20 d, which seems to correspond to our results; but his reactor operated at 45°C and at 15% maltodextrin (*5*). It may be suspected that a 30% substrate concentration would have provoked the washout of these light particles, which do not wash out with magnetic particles because of their higher density.

CONCLUSION

In the present work, the continuous production of glucose syrups in a recycled FBR containing immobilized GA, a project that often aborted for economical reasons, has been estimated to be viable since critical aspects of this technique have been improved. The magnetic microparticle used is cheap and easy to handle and manufacture. GA is tightly fixed on the support and has a high remaining activity and a high stability after immobilization and crosslinking of its carbohydrates and after, the continuous hydrolysis of maltodextrin in the fluidized bed reaches a high conversion due to the plug-flow behavior of the reactor. The system described here is appropriate for the production of high-conversion syrup. However, for the production of high-dextrose syrup, the bed height and the particle loading should be increased to allow acceptable residence times and initial flow rates.

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