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Stability of Immobilized Amyloglucosidase in the Process of Cassava Starch Saccharification

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ABSTRACT

The half-life of immobilized amyloglucosidase was determined in a fluidized-bed reactor operating continuously with a 30% w/v liquefied cassava starch solution at pH 4.5 and temperatures from 50 to 70°C. For the higher temperatures: 60, 65, and 70°C, thermal deactivation gives half-lives of 127, 38 and 7.3 h, respectively, in close agreement with corn starch data. For the lower temperatures: 55 and 60°C, the deposition of impurities over the immobilized enzyme particle contributes significantly to deactivation, lowering expected half-lives to 32.6 and 13.2 d, respectively. Commercial exploitation of this process would then require low temperature of operation, thorough purification of the substrate solution, and control of microbial contamination to achieve sufficiently long half-lives.

Index Entries: Cassava starch; amyloglucosidase; stability; immobilized enzyme.

INTRODUCTION

Heterogeneous catalysts of an equivalent nature to classical inorganic catalysts that are largely used today in the chemical industry can be produced by immobilization of enzymes onto supports that are insoluble in their substrate solution. These modern heterogeneous catalysts stand out because of two characteristics they inherit from their enzymatic nature: (1) very high substrate specificity, and (2) operational conditions that are close to ambient conditions (*1,2*).

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Currently, the main industrial applications of immobilized enzymes are in the following processes:

1. High-fructose syrup;
2. Semisynthetic penicillin; and
3. The processing of cheese whey (*2,3*)

The fixed-bed reactor model has been chosen for all of these applications. However, other reactor configurations have been developed, and there is considerable interest in the application of the fluidized-bed model owing to its various advantages (*4–6*).

Commercial success in the application of immobilized enzymes depends on its initial activity and stability. Highly active preparations, but with low stability can be tolerated if the support is cheap and available in abundant supply or can be easily regenerated. In such a case, it can then be frequently changed at low cost. However, immobilized enzymes of high stability are required when the enzyme or the support are expensive, or regeneration is troublesome because frequent substitution of the immobilized enzyme would then be economically prohibitive.

An immobilized enzyme process will only be more economical than the equivalent processes that use soluble enzyme, if the immobilized enzyme has a sufficient long half-life. The cost benefit derived from reduction in enzyme consumption should be great enough to compensate for the additional expenses with the immobilization process (*7,8*).

To study the economical feasibility of producing glucose from cassava starch using immobilized enzyme technology, a general program of research was initiated some years ago in our department. This work reports on the half-life of immobilized amyloglucosidase used in a fluidized-bed reactor. This was operated continuously with liquefied cassava starch (30% w/v, pH 4.5) at various test temperatures ranging from 50 to 70°C. These conditions were chosen because they comprehend the operational conditions of the envisaged immobilized enzyme cassava starch saccharification process. The study of design alternatives and process optimization requires knowledge on immobilized amyloglucosidase half-life as a function of temperature in actual process conditions.

MATERIALS AND METHODS

Materials

Substrate

Aqueous cassava starch (COPAGRA-PR) suspension was liquefied with α-amylase (Termamyl 120L, NOVO) in the presence of 70 ppm of $CaCl_2$, pH 6.0, and temperature of 90–100°C in a period of 1 h. Acetate buffer (pH 4.5, 2*M*) was added to this solution in the proportion of 1% of the final volume. This was adjusted with water to give the desired

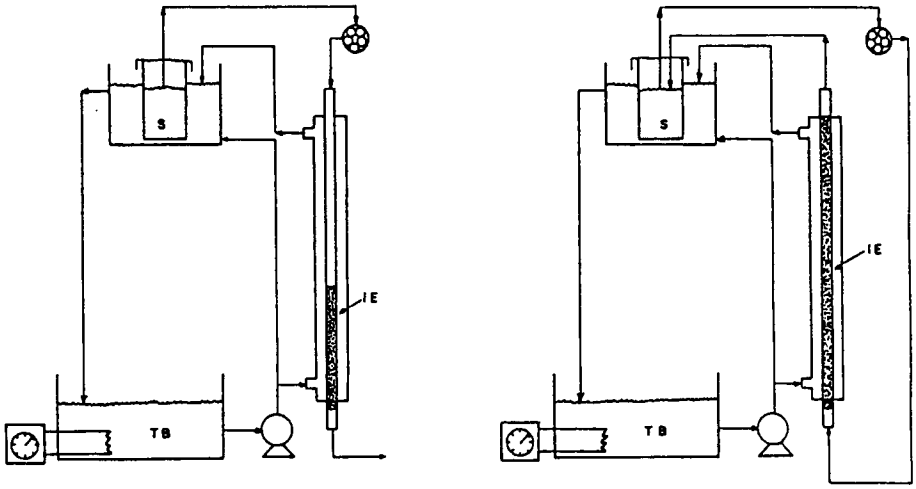

Fig. 1. Reactor experimental setup for studying immobilized enzyme half-lives.

maltodextrin concentration of 30% w/v. The solution also contained 0.1% w/v of sodium benzoate to retard microbial growth (*9*).

Enzyme

Aspergillus niger amyloglucosidase, kindly donated by NOVO (AMG 150L, with 130.5 mg of protein/mL) was immobilized into controlled-pore silica (CPS, Corning Glass) by the silane-glutaraldehyde method (*7,8*). Mean particle diameter of the CPS and its average pore size were 0.436 and 37.5 nm, respectively. The initial activity of the immobilized AMG, determined in the substrate solution at 45°C, was 120.5 U/g of dry support and 22.62 U/mg of protein (*9,10*). One unit of enzyme activity corresponds to the production of 1 μmol of glucose/min using the conditions specified.

Reactor

The reactor was constructed using a glass tube with an internal diameter of 7.5 mm and a useful height of 380 mm. The reactor had an external jacket in which water at constant temperature was recirculated to maintain isothermal conditions. The liquid distributor consisted of a fine stainless-steel mesh pressed into spherical form (*11*). The experimental setup is diagrammatically shown in Fig. 1.

Methods

Half-Life

Immobilized enzyme (IE) half-life was measured by determining the time required for the IE activity to decay to 50% of its initial value. The conversion of cassava starch to glucose as it passed through the reactor was used to describe the relationship between IE activity decay and time.

Conversion

Conversion (X_A) of the substrate solution into glucose was calculated using the glucose concentration values determined in samples taken at the reactor inlet and outlet.

$$X_A = 100\, f\, (C_g - C_{gi}) / (C_{Ao} - f\, C_{gi}) \quad (1)$$

where C_{Ao} = initial starch concentration, 300 mg/mL, C_g = glucose concentration, mg/mL, C_{gi} = initial glucose concentration, mg/mL, and f-ratio of molecular weights for the anhydroglucose unit in the starch molecule and glucose, f = 162/180 = 0.9.

Glucose

Glucose concentration was assayed by the *ortho*-toluidine method (*12*).

Reactor Conditions

Conversion of cassava starch to glucose within the reactor was tested using temperatures of 50, 55, 60, 65, and 70°C. The immobilized AMG load in the reactor was chosen to give a maximum initial conversion of $< 10\%$, so as to be within the range of linearity between conversion and total IE activity. Liquid superficial velocity inside the reactor was set to 7 mm/s, which is suitable for operating the reactor either as a fixed bed with downflow or as a fluidized bed with upflow.

Reactor Operational Modes

The reactor was operated continuously in cycles of a fixed-bed mode with single pass, followed by a fluidized-bed mode with recycle. The fixed-bed mode was used only while determining reactor conversion (periods of 1 h), whereas the fluidized mode was used for completing the cycle periods. The cycle periods used were 2, 12 and 24 h for the tests conducted at 70, 65, and 60-55-50°C, respectively. For each new cycle the substrate solution was changed. The total operational time of each test was determined by the rate of activity decay, which is faster for the higher temperatures. The test was continued up to the point where the IE activity dropped to about 25–30% of its initial value. For the lower temperature, sanitization of the reactor was performed at the end of each cycle using 40 mL of substrate solution containing 0.05% v/v of formaldehyde. More details about these methods are given by Zanin (*9*).

RESULTS AND DISCUSSION

The data concerning conversion as a function of immobilized enzyme mass shown on Fig. 2 demonstrate that with all temperatures there was a linear relation between total enzyme activity in the reactor and conversion. These graphs served the purpose of determining the relative residual activity of the enzyme at each cycle (*see* Methods).

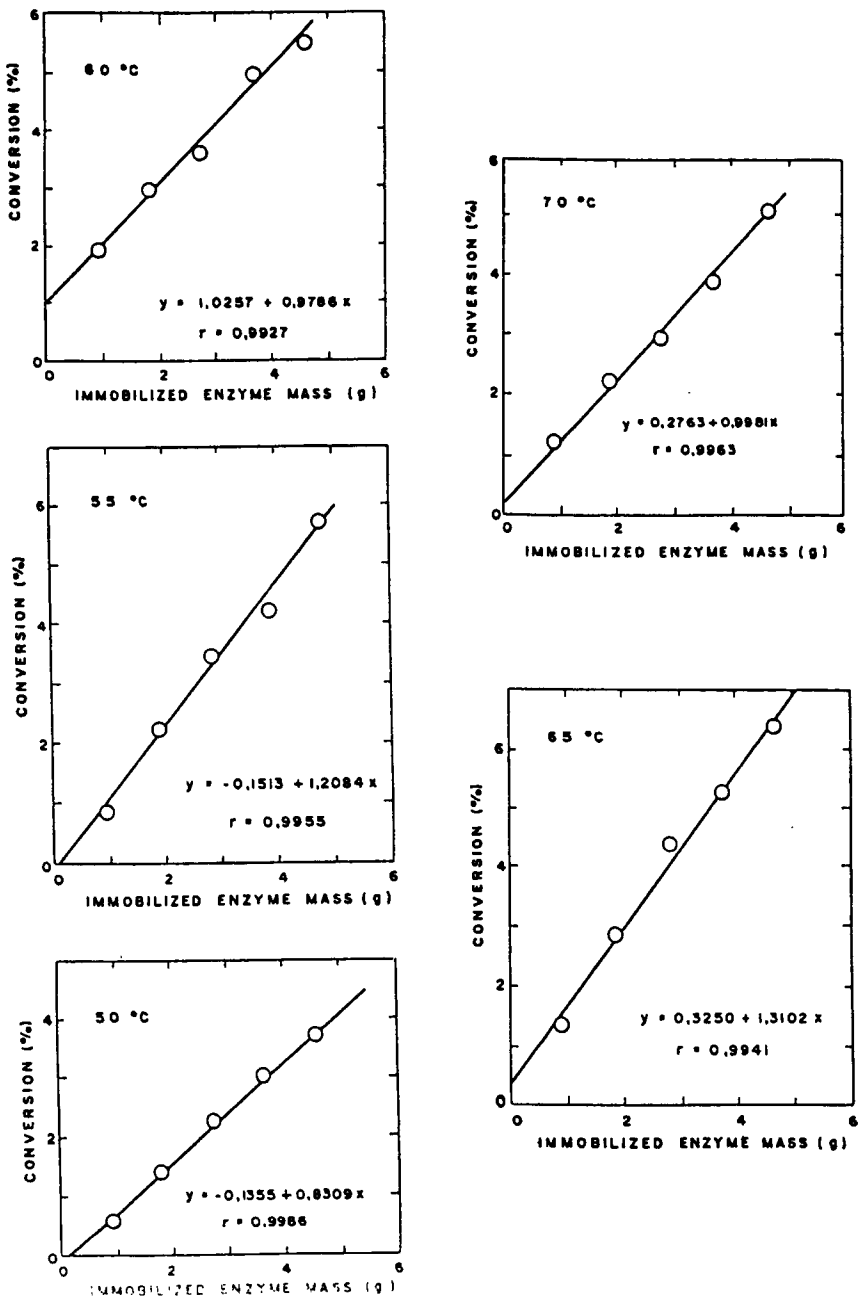

Fig. 2. Conversion of the liquefied starch substrate solution into glucose as a function of the immobilized amyloglucosidase loaded into the fixed-bed reactor. Data for tests conducted at temperatures from 50 to 70°C.

The exponential decay model was adjusted to the data on immobilized enzyme residual activity (A_R) as a function of the time in operation (t) (*see* Fig. 3), producing the following equations:

$$70°C: A_R = 99.3 \exp(-9.432 \times 10^{-2} t),\ r = 0.9912 \quad (2)$$

$$65°C: A_R = 94.5 \exp(-1.680 \times 10^{-2} t),\ r = 0.9931 \quad (3)$$

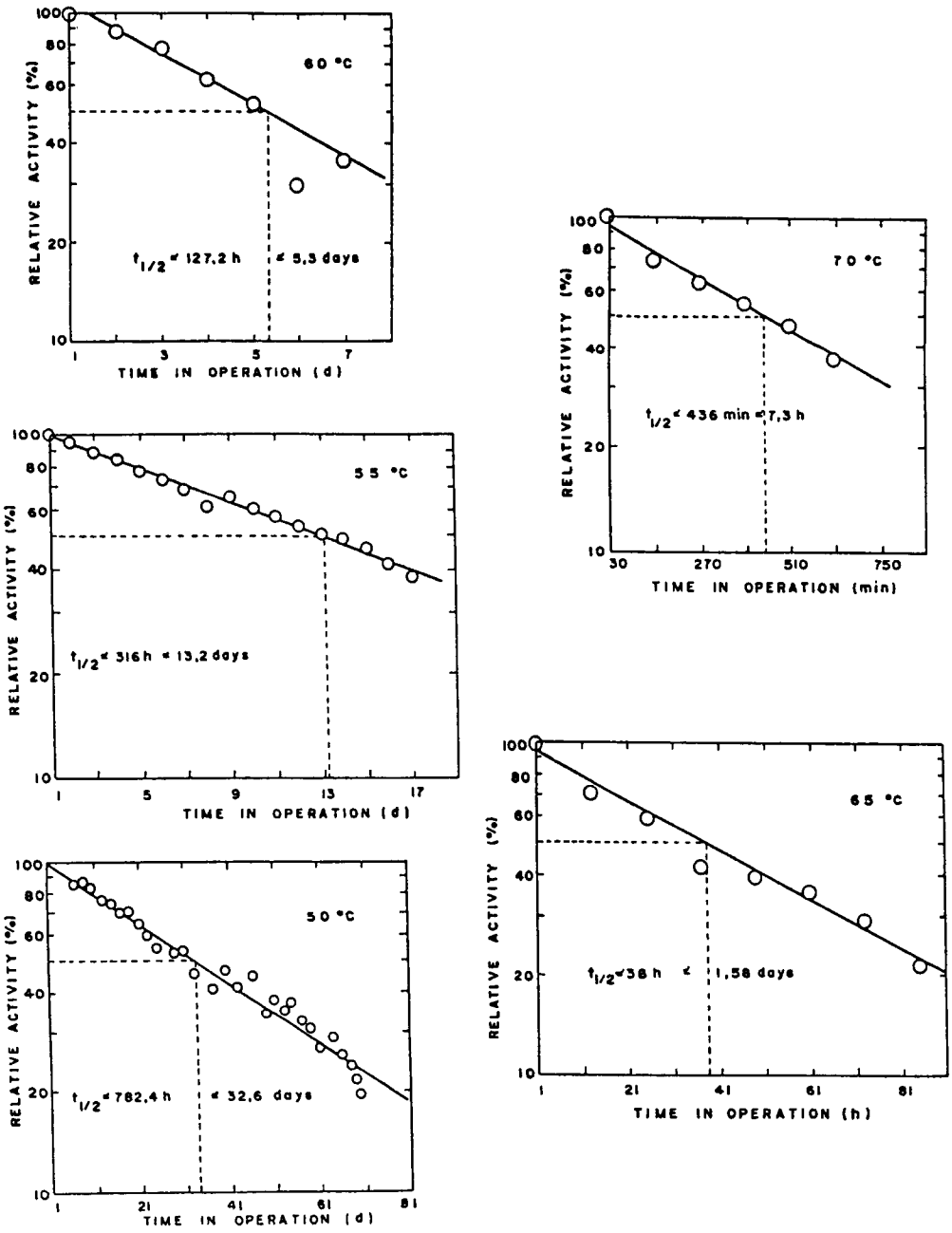

Fig. 3. Residual activity of the immobilized amyloglucosidase as a function of the elapsed time of continuous operation of the fluidized-bed reactor. Data for tests conducted at temperatures from 50 to 70°C.

$$60°C: A_R = 124.0 \exp(-7.121 \times 10^{-3} t),\ r = 0.9954 \quad (4)$$

$$55°C: A_R = 105.8 \exp(-2.371 \times 10^{-3} t),\ r = 0.9967 \quad (5)$$

$$50°C: A_R = 97.0 \exp(-8.458 \times 10^{-4} t),\ r = 0.9845 \quad (6)$$

A very reasonable fit is observed suggesting that the main process of enzyme deactivation is thermal. This is also true in the case of free enzyme. If the main deactivation process was limited by diffusion, a nonexponential decay would be expected (*13*). A greater degree of scatter can be observed for the data on residual activity at 50°C. This was probably because of incipient microbial contamination detected over the longer periods of time that is required for conversion at lower temperatures.

The half-lives determined with Eqs. (2)–(6) are shown in Fig. 4 and Table 1. The latter also presents data from Lee et al. (*14,15*), who used similar experimental conditions taking corn starch as the substrate instead. Good agreement can be observed for the half-lives obtained at temperatures of 70, 65, and 60°C. Weetal and Havewala (*16*) extrapolated data published by Lee et al. (*14,15*) to include the conversion temperature of 45°C. A half-life of 645 d was calculated. Our own data extrapolated to the same temperature give 582 d. However, we found that the half-lives for the lower temperatures were lower than expected from the extrapolated values. For example, Fig. 4 and Table 1 show that the expected half-lives at 55 and 50°C were of 25.5 and 119 d, respectively, whereas only 13.2 and 32.6 d were obtained in practice.

It seems that what reduces immobilized enzyme half-life is a phenomenon that is similar to the problems of fouling during classical inorganic heterogeneous catalysis. Cassava liquefied starch still contains small colloidal micelles that settle down very slowly. These and other impurities seem to be deposited onto immobilized enzyme particles, because their color is observed to change with time (*9*). The nature of these micelles and impurities has not been determined. However, it is believed that the micelles may be constituted of either retrograded starch, proteins, or other polysaccharides other than starch that are present in very small quantities in the substrate solution. These deposits may hinder the contact between substrate and immobilized enzyme, therefore reducing overall activity with time. This constitutes a parallel path for immobilized enzyme deactivation other than thermal denaturation. The process is slower than the latter and hence only observed at lower temperatures that are associated with longer half-lives. Cheetham (*13*) also comments on the deposition of impurities over immobilized enzyme particles.

It becomes clear then that a thorough purification of the substrate solution is required to reach longer half-lives. This additional step lowers the economical advantage of using an immobilized enzyme process in such a way that it is not substantiated in the literature.

The half-life of 5.3 d so far obtained for immobilized amyloglucosidase at 60°C, a temperature that many industrialists would prefer, to avoid contamination problems, does not encourage as yet the commercial implementation of immobilized enzyme saccharification of cassava starch. The use of lower temperatures (e.g., 45°C) would give desirable half-lives on the order of 50 d (*9*), but would first require the development of safe and economical means to control microbial contamination.

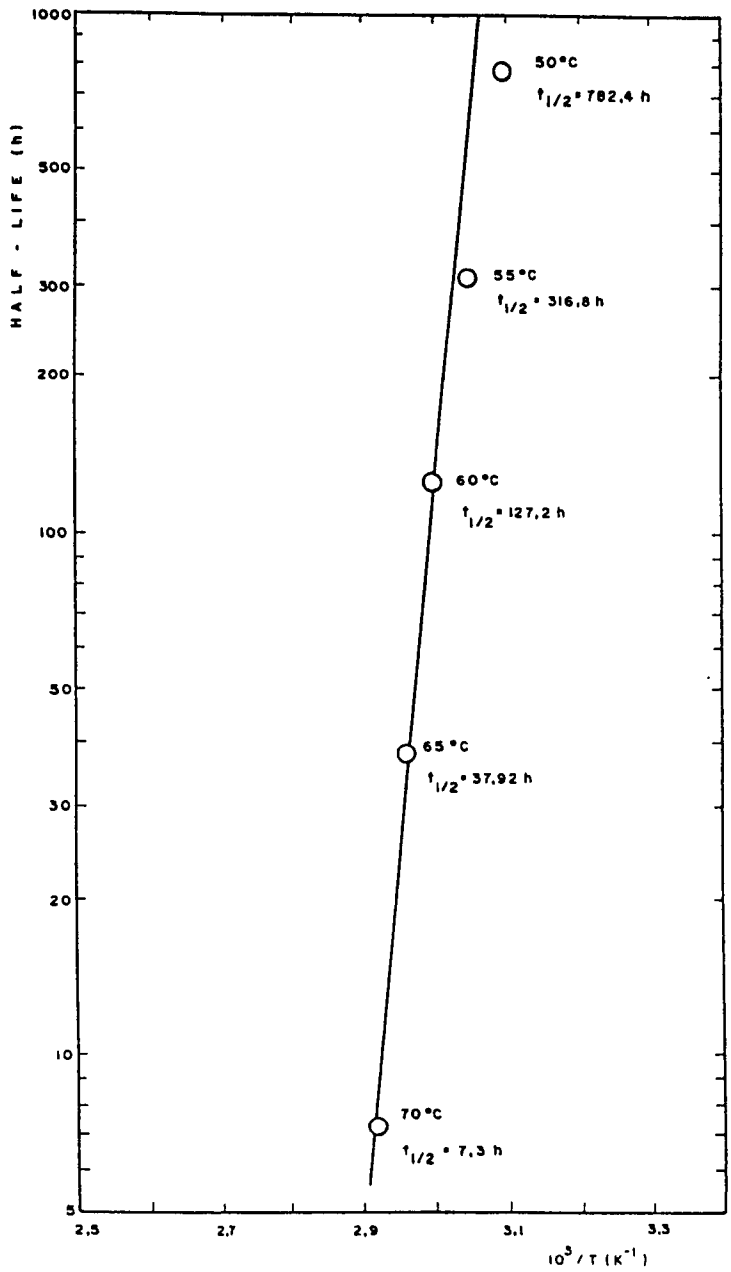

Fig. 4. Immobilized amyloglucosidase half-life as a function of temperature for the saccharification of liquefied cassava starch (30% w/v, pH 4.5).

CONCLUSIONS

1. For temperatures of 70, 65, and 60°C, the deactivation of immobilized amyloglucosidase fluidized by 30% w/v liquefied cassava starch, pH 4.5, is mainly thermal, giving the half-lives of 7.3 h, and 1.6 and 5.3, d respectively. These results are very close to those published for corn starch.

Table 1
Experimental and Estimated Enzyme Half-Lives

Temp., °C	This work			Lee et al. (1975)		Lee et al. (1976)	
	Hours	Days	Fitted value days	Hours	Days	Hours	Days
70	7.3	0.30	0.33	7.5	0.31	7.5	0.31
65	37.9	1.58	1.34	34.7	1.45	34.7	1.45
60	127.2	5.30	5.73	117.6	4.90	117.5	4.90
55	316.8	13.2	25.5	519.0	21.6	581	24.2
50	782.4	32.6	119	2180[a]	90.8	2550[a]	106.3
45	—	—	582	8800[a]	367	12,200[a]	508

[a]Estimated.

2. For the lower temperatures, 55 and 50°C, in addition to the thermal deactivation, fouling of the immobilized enzyme particles becomes important. This phenomenon lowers expected half-lives from 25.5 and 119 d to only 13.2 and 32.6 d, respectively.
3. To obtain higher half-lives using the immobilized enzyme process, it may be necessary to include additional steps for the purification of the substrate solution. This would raise process costs, reducing its economical advantages.
4. The half-life of 5.3 d obtained at 60°C is insufficient for commercial application of immobilized AMG. The use of lower temperatures, such as 45°C, for which practical half-lives on the order of 50 d would be obtained, is possible, if contamination can be controlled economically.

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