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Immobilization of *Aspergillus niger* NRC 107 Xylanase and β-Xylosidase, and Properties of the Immobilized Enzymes

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ABSTRACT

Aspergillus niger NRC 107 xylanase and β-xylosidase were immobiliized on various carriers by different methods of immobilization, including physical adsorption, covalant binding, ionic binding, and entrapment. The immobilized enzymes were prepared by physical adsorption on tannin-chitosan, ionic binding onto Dowex-50W, covalent binding on chitosan beads through glutaraldehyde, and entrapment in polyacrylamide had the highest activities. In most cases, the optimum pH of the immobilized enzymes were shifted to lower than those of free enzymes. The optimum reaction temperature of immobilized xylanase was shifted from 50°C to 52.5–65°C, whereas that of immobilized β-xylosidase was shifted from 45°C to 50–60°C. The K_m values of immobilized enzymes were higher than those of native enzymes. The operational stability of the immobilized enzymes was evaluated in continuous operation in packed-bead column-type reactors. The enzymes covalently bounded to chitosan showed the highest operational stability. However, the enzymes immobilized by physical adsorption or by ionic binding showed a low operational stability. The enzymes entrapped in polyacrylamide exhibited lower activity, but better operational stability.

Index Entries: *Aspergillus niger;* immobilized xylanase; immobilized β-xylosidase; xylan hydrolysis; packed-bead reactors.

INTRODUCTION

Hemicellulose is a heterogeneous polysaccharide comprising 20–40% of agricultural and foresty residues. The hydrolysis of its characteristic backbone, consisting of β-1,4-linked D-xylosyl residues, involves β-xylanase (1,4-β-D-xylan xylanohydrolase, EC 3.2.1.8) and β-xylosidase (1,4-β-xylan xylohydrolase, EC 3.2.1.37) (*1*).

Xylanases degrade plant xylans to xylose, and xylo-oligosaccharides have received much attention on account of industrial use of xylose as a source of xylitol for use as a sweetener. In addition, hydrolysis products of xylan can also be used for production of various fermented products (*2,3*). Furthermore, the special application of xylans in cellulose pulping (*4,5*) and preparing other lignocellulosic fibers (*6*) has been reported.

For industrial application, the immobilization of enzymes on solid support can offer several advantages, including repeated usage of enzyme, ease of product separation, improvement of enzyme stability, and continuous operation in packed-bead reactors. As far as xylanases are concerned, however, there are few reports about the immobilization of xylanases (*7–11*).

In previous work, an active extracellular cellulase-free xylanase was produced by the *Aspergillus niger* strain (*12*). This enzyme was successfully used for enzymatic hydrolysis of hemiculluloses from different agricultural wastes in bioconversion more than 88% (*13*). However, this method is not suitable for practical application, since it requires a large amount of the enzyme. In addition, using higher concentrations of the substrate (more than 12%) showed a negative effect on the enzyme recovery.

This work describes the immobilization of *Aspergillus niger* xylanase and β-xylosidase onto various carriers using physical adsorption, covalent binding, ionic binding, and entrapment. The properties of the immobilized enzymes were compared with those of free enzyme. In addition, the performance of immobilized enzymes in continuous operation in packed-bead reactor will be discussed.

MATERIALS AND METHODS

Preparation of Hemicellulase Enzyme

Hemicellulase enzyme preparation was obtained from *Aspergillus niger* NRC 107 by the method described in a prevous paper (*12*). The culture filtrate was concentrated by ultrafiltration through a Diafilter of G-10T (Bio-Engineering Co., cutoff mol wt, 10,000). The concentrated culture filtrate was fractionated by 80% saturation with ammonium sulfate. This partially purified enzyme (specific activities: xylanase 88.6 U/mg protein, β-xylosidase 7.46 U/mg protein) was used for the preparation of the immobilized enzyme.

Carriers

These include porous glass (controlled pore, 730 Å, 20–40 mesh) and ceramics from NGK Insulators Ltd., Nagoya, Japan, Sepharose CL-4B, CM, SP, and DEAE-Sephadex, from Pharmacia Fine Chemicals Inc., Uppsala, Sweden, Amperliet IRC-50, and IRA-400C, Dowex WGR-2, and 50W. Chitin and chitosan were from Sigma Chemical Co., USA. Chitopearl (BCW 1010, BCW 2510, BCW 3010, BCW 3520), a porous chitosan bead crosslinked with aliphatic or aromatic compound, was suppled by the Fuji Spinning Co Ltd., Japan. All other chemicals were of analytical grade.

Substrates

p-Nitrophenyl-β-D-xylopyranoside (PNPX) was supplied by Sigma Chemical Co, USA. Larch wood xylan was obtained from Aldrich Chemical Co. Inc.

Immobilization Methods

Physical Adsorption

One hundred milligrams of the carriers were incubated with the enzyme solution (250 U xylanase, 21 U β-xylosidase) dissolved in a 3 mL of 0.05*M* acetate buffer (pH 5.0) at 4°C over night. The unbound enzymes were removed from the carriers by washing three times with 0.05*M* acetate buffer containing 0.1*M* NaCl.

Ionic Binding

Cation or anion exchanger (1 g) equilibrated with acetate buffer (0.05*M*, pH 3–6) or phosphate buffer (0.05*M*, pH 7–8) was incubated with 3 mL of enzyme solution (500–1250 U xylanase, 42–105 U β-xylosidase) dissolved in the same buffer at 4°C for 24 h. The unbound enzymes were removed as described above.

Covalent Coupling

Aminohexyl Sepharose (AH-Sepharose) was prepared according to the procedures of Matsumoto et al. (*14*) and Watanabe et al. (*15*). One gram of the carrier ws incubated with shaking (4°C for 24 h) with 5 mL of the enzyme solution (2500 U xylanase, 210 U β-xylosidase) dissolved in 0.05*M* acetate buffer (pH 5.6) containing 36 mg of carbodiimide reagent [1-cyclohexyl-3-(2-morpholinoethyl)carbodiimidemetho-*p*-toluenesulfonate]. The unbound enzymes were removed as described above.

Cyanogen bromide-activated Sepharose (CNBr-Sepharose) was prepared by the method of March et al. (*16*). One gram of the carrier was incubated at 4°C for 24 h with shaking with the enzyme solution (2500 U xylanase, 210 U β-xylosidase) dissolved in 5 mL of 0.05*M* phosphate buffer (pH 7.0). The unbound enzymes were removed as described above.

Aminoalkylsilane-alumina (AS-alumina) was prepared by the method of Weetall (*17*) and was treated with glutaraldehyde by the method of Dixon et al. (*18*). The enzyme solution (2500 U xylanase, 210 U of β-xylosidase) dissolved in 5 mL of 0.05*M* phosphate buffer (pH 5.6) was incubated with the shaking of 1 g of the carrier at 4°C for 24 h. The unbound enzymes were removed as described above.

Chitosan beads were prepared as follow: One gram of chitisan was shaken in 5 mL of 0.05*M* acetate buffer (pH 5.0) containing 2.5% glutaraldehyde (GA) for 1 h at 30°C. Chitosan beads were collected by filteration and washed with deionized water to remove excess GA. The wet chitosan beads were mixed with the 5 mL of the enzyme solution (5000 U xylanase, 420 U β-xylosidase). After being shaken for 1 h at 30°C, unbound enzymes were removed by washing with deionized water.

Entrapment

In Polyacrylamide

The gel was prepared according to the method of Roy et al. (*9*). The polymerization mixture contained 10 mL of the enzyme solution (10000 U xylanase and 840 U β-xylosidase), 50 mL of 13.6% acrylamide, and 40 mL of Tris-HCl buffer (10 m*M*, pH 7.5). The amount of crosslinking monomer (*N,N*-methylene-*bis*-acrylamide) added was at the levels of 2, 4, 6, and 8% of the total monomer content in separate experiments. The catalyst system consisted of the following: 0.2 mL of *N,N,N,N*-tetramethylene-diamine and 0.1 g of potassium persulfate dissolved in 2 mL of distilled water. It was finally added to the acrylamide mixture, and the stirring was continued for 5 min. The total volume of the mixture was kept to 100 mL with deionized water. Finally, the mixture was transferred to the gel-casting glass cassettes for polymerization. The glass cassettes were kept in a glass chamber with N_2 atmosphere at 30°C. After the polymerization the, gel was washed with saline, cut into 1-mm fragments, and kept in Tris-HCl buffer (10 m*M*, pH 7.5) at 4°C for 72 h to remove the unbound enzymes.

In Ca-Alginate

In separate experiments, 100 mL of different concentrations of sodium alginate solution (4, 6, 8, and 10%) were mixed with 10,000 U xylanase and 840 U β-xylosidase. The entrapment was carried out by dropping the alginate solutions in 100 m*M* CaCl solution. The resulting beads (0.5–1.0 mm diameter) were collected, washed with acetate buffer (0.05 *M*, pH 5.0), and kept in the same buffer at 4°C for 72 h to remove the unbound enzymes.

Determination of Enzyme Activities

Xylanase activity was determined by incubating 0.5 mL of 1.0% (w/v) larch wood xylan in 0.05*M* acetate buffer (pH 5.0) and a weighed sample of immobilized enzyme at 45°C for 30 min. The formed reducing sugars were determined by the method of Somogyi (*19*). One unit of xylanase

activity was defined as the amount of enzyme liberating 1 μmol of reducing sugars as a xylose/min.

β-Xylosidase activity was also assayed by measuring the amount of *p*-nitrophenol liberated from *p*-nitrophenyl-β-D-xyloside (PNPX). The assay mixture consisted of 0.5 mL of acetate buffer (0.05*M*, pH 5.0), 0.5 mL of 0.05*M* PNPX, and weighed amount of immobilized enzyme. The reaction mixture was incubated at 45°C for 15 min, and then 2 mL of Na_2CO_3 were added to stop the reaction. The color that developed was read at 400 nm. One unit of β-xylosidase activity is defined as the amount of enzyme that produces 1 μmol of *p*-nitrophenol/min.

Operating Stability of Immobilized Enzymes in a Packed-Bead Reactor

The column reactors was prepared by packing each immobilized enzyme (20 g, wet) in a glass jacket column (1 cm diameter). The jacket was kept at the optimum temperature of the immobilized xylanase. The larch wood xylan suspension (5%, w/v) was heated at 100°C for 5 min in a boiling water bath giving a transluant solution. This solution was diluted with 0.05*M* acetate buffer (at the optimum pH of each enzyme) to a final concentration of 1%, w/v. This substrate solution was supplied to the reactors at a flow rate of 30 mL/h. The reducing sugars that were formed in the effluent were determined by the method of Somogyi (*19*) as xylose.

Analytical Methods

The protein of free and immobilized enzymes was measured by the method of McGrath (*20*) with leuocine as a standard. Reducing sugars were determined by the method of Somogyi (*19*) with xylose as a standard.

RESULTS AND DISCUSSION

Immobilization by Physical Adsorption

A. niger xylanase and β-xylosidase were immobilized by physical adsorption on different carriers (Table 1). One of the most important factors controlling immobilization by physical adsorption on porous carriers is the carrier pore size (*21*). The pore diameters of the carriers used (ceramics, chitosan, porous glass, and Sepharose) were 400–2000 Å. On the other hand, the pore diameters of *A. niger* xylanases and β-xylosidases were calculated to be 40 and 60 Å, respectively (*22*). From here on, the carriers used have pore sizes large enough to accommodate the two enzymes. However, it was observed that the yield of immobilized β-xylosidase (low-molecular-weight substrate) was greater than the yield of xylanase (high molecular weight substrate). These results suggested that the suitable pore size of the carrier depends on the molecular weight of both the enzyme and the substrate. These findings coincide with those

Table 1
Immobilization of *A. niger* NRC 107 Xylanase and β-Xylosidase by Physical Adsorption

	Added enzyme, A, U/g		Unbound enzyme, B, U/g		Immobilized enzyme I, U/g		Yield = I/(A − B)%	
Carrier	XA[a]	XB[b]	XA	XB	XA	XB	XA	XB
Ceramics	2500	210	1600	106	88.6	66.0	9.80	23.14
Chitin	2500	210	970	102	212.0	25.0	13.80	23.14
Chitosan	2500	210	1116	56	412.0	184.0	29.70	66.60
Porous glass	2500	210	1430	116	109.6	48.6	10.24	51.70
Tannin-Sepharose	2500	210	1030	90	229.0	70.8	16.60	59.00
Tannin-chitosan	2500	210	912	109	600.0	70.0	37.70	69.30

[a]XA: Xylanase activity.
[b]XB: β-xylosidase activity.

reported by Lomako et al. (23), who reported that the hydrolyzing activity of glucoamylase adsorbed on porour glass depends on the pore size, but its maltose hydrolyzing activity was independent of this. The enzyme immobilized on Tannin-chitosan showed the highest immobilization yield and the highest immobilized units per gram. Therefore, xylanase and β-xylosidase immobilized on Tannin-chitosan were taken as typical examples for immobilization by physical adsorption.

Immobilization by Covalent Binding

All the immobilized xylanase and β-xylosidase through covalent binding had a considerably good activity, especially chitosan (Table 2). This may be attributed to the formation of stable crosslinking between the enzyme and the support. In addition, covalent binding of the enzyme to the support through a spacer group probably increased the local surface area of the support and, consequently, reduced the steric hindrance in the immediate vicinity of the enzyme molecule. In the following experiments, chitosan was used as a typical example for immobilization by covalent binding.

Immobilization by Ionic Binding

The data on the immobilization of xylanase and β-xylosidase by ionic binding to various ion exchangers are summarized in Table 3. In general, immobilization by ionic binding showed low immobilization yield. Comparatively, Dowex-50W was the most suitable ion exchanger for the enzymes' immobilization. Since the immobilized enzymes had the highest activity per gram support and the highest immobilization yield, they were therefore selected as typical examples for this group.

Table 2
Immobilization of *A. niger* NRC 107
Xylanase and β-Xylosidase by Covalent Binding

	Added enzyme, A, U/g		Unbound enzyme, B, U/g		Immobilized enzyme I, U/g		Yield = I/(A − B)%	
Carrier	XA[a]	XB[b]	XA	XB	XA	XB	XA	XB
AH-Sephadex	2500	210	470	30	480	50.0	23.6	27.7
AS-Alumina	2500	210	1180	58	570	86.3	43.1	56.7
CNBr-Sepharose	2500	210	820	53	470	34.0	28.0	21.6
Chitosan	5000	420	2730	120	1270	222.0	55.9	74.0
Chitopearl								
BCW 1010	5000	420	2789	202	577	119.0	26.0	54.6
BCW 2510	5000	420	2670	179	699	174.0	30.0	72.2
BCW 3010	5000	420	2400	169	890	177.0	34.2	70.8
BCW 3520	5000	420	2100	150	969	167.0	33.4	61.8

[a]XA: Xylanase activity.
[b]XB: β-xylosidase activity.

Table 3
Immobilization of *A. niger* NRC 107
Xylanase and β-Xylosidase by Ionic Binding

	Added enzyme, A, U/g		Unbound enzyme, B, U/g		Immobilized enzyme I, U/g		Yield = I/(A − B)%	
Carrier	XA[a]	XB[b]	XA	XB	XA	XB	XA	XB
Amberlite IEC-50	500	42	118	11	42	8.6	12.3	27.7
Amberlite IRA-400C	500	42	180	14	27	10.3	8.4	36.7
Dowex 50-W	500	42	312	11	115	14.7	24.5	47.4
Dowex WGR-2	500	42	147	13	47	10.7	13.3	36.9
CM-Sephadex	1250	105	403	17	33	9.4	3.8	22.0
SP-Sephadex	1250	105	370	19	51	22.6	5.8	26.2
DEAE-Sephadex	1250	105	317	35	62	17.3	6.6	24.7
DEAE-Celluluose	1000	84	418	14	81	18.6	13.9	26.5

[a]XA: Xylanase activity.
[b]XB: β-xylosidase activity.

Immobilization by Entrapment

The results of immobilization of xylanase and β-xylosidase by entrapment are shown in Table 4. The results suggested that gradually increasing the level of crosslinking of polyacrylamide gel up to 6% resulted in an increase in entrapping efficiency and the immobilization yield. Likewise, there was a parallel relationship between the concentration of Ca-alginate

Table 4
Immobilization of *A. niger* NRC 107
Xylanase and β-Xylosidase by Entrapment

Carrier	Added enzyme, A, U/10 mL gel		Unbound enzyme, B, U/10 mL gel		Immobilized enzyme I, U/10 mL gel		Yield = I/(A − B)%	
	XA[a]	XB[b]	XA	XB	XA	XB	XA	XB
Crosslinking of polyacrylamide gel[c]								
2%	1000	84	279	38.6	136.6	17.0	18.65	25.37
4%	1000	84	190	31.4	174.0	25.8	21.50	49.05
6%	1000	84	154	24.0	233.6	32.5	27.54	54.33
8%	1000	84	119	18.0	212.0	30.4	24.06	46.06
Ca-alginate concentration								
4%	1000	84	396	39.3	89.0	17.0	14.73	28.00
6%	1000	84	303	32.4	141.0	24.0	16.40	46.00
8%	1000	84	262	28.6	156.0	27.0	21.00	49.27
10%	1000	84	188	24.3	184.0	30.3	22.60	50.73

[a]XA: Xylanase activity.
[b]XB: β-xylosidase activity.
[c]Total concentration of polyacrylamide was 7%.

and the entrapped enzyme. However, it was observed that Ca-alginate was inapplicable to the enzyme immobilization because of the leak out of xylanase from the gel, even at 10% Ca-alginate concentration (not shown data). In addition, apart from working with higher gel concentrations (especially at room temperature, 24°C), the mixing of the enzyme is very difficult. In the following experiments, xylanase and β-xylosidase entrapped in polyacrylamide (6% crosslinking) were used.

Properties of Immobilized Enzymes

The immobilized enzymes retain 15–25% of specific xylanase activity and 16–33% of specific β-xylosidase activity (Table 5). This drop in the specific activity can be attributed to steric hindrance in the immediate vicinity of the enzyme molecules. These hindrances are probably caused by the shielding effect of the substrate and by the excessive packing of the enzyme, which render their active sites less accessible to the substrate (*24*). Multiple fixation of the enzyme to the matrix would also lead to a decrease in catalytic activity owing to the decrease in flexibility of the enzyme molecule. Since the immobilization showed greater loss of specific activity (especially xylanase) enhance the stability of the enzyme (*see below*). This mechanism may give a plausible explanation for the reduced specific activity. However, it is difficult to distinguish between these effects and the reduced diffusivity of the substrate in the porous medium. This in particularly so with a macromolecular substrate, like xylan.

Table 5
Properties of Immobilized Enzymes

Properties	Free enzyme	Carrier of immobilized enzyme			
		Tannin-chitosan	Dowex-50W	Chitosan	Polyacrylamide
Xylanase activity (XA)					
Specific activity (U/mg protein)	88.60	21.46	20.6	17.94	13.5
Optimum pH	5.0	4.2	3.8	4.0	4.2
Optimum temperature	50.0	52.5	54.0	62.0	65.0
Thermal stability at pH 5, for 60 min	55.0	50.0	48.0	60.0	66.5
K_m (mg/mL)	1.33	3.24	3.20	4.80	5.9
V_{max} (μmol/mg protein/min)	186.0	56.8	22.4	37.9	19.5
β-xylosidase activity (XB)					
Specific activity (U/mg protein)	7.46	1.84	0.96	2.44	1.20
Optimum pH	4.5	4.2	3.8	4.0	4.4
Optimum temperature	45.0	50.0	50.0	58.0	60.0
Thermal stability at pH 5, for 60 min	55.0	54.0	50.0	62.0	66.0
K_m (mM)	1.5	2.94	3.75	2.25	4.25
V_{max} (μmol/mg protein/min)	9.4	2.94	2.65	3.75	0.97

The optimum pH of the immobilized enzymes was shifted to acidic range in comparison to the free enzyme (Table 5). These effects may be dependent on the ionic environment around the active site of the enzyme bound to the carrier. Similar shifts of optimum pH were reported for other immobilized xylanases and β-xylosidases (*10,11*).

The optimal reaction temperature shifted from 50°C for free xylanase to 52.5–54°C for the enzyme immobilized on Tannin-chitosan (physical adsorption) and Dowex-50W (ionic bonding). A greater evaluation in optimal temperature was found with the enzymes immobilized on chitosan by covalent binding (58°C for β-xylosidase, 62°C for xylanase) or the enzyme entrapped in polyacrylamide (60°C for β-xylosidase, 65°C for xylanase). These results indicate that xylanase and β-xylosidase were more stable when they were immobilized with crosslinking or by entrapment in polyacrylamide. In addition, the enzymes immobilized by physical adsorption or by ionic binding were released from the carrier at a higher temperature (more than 60°C). These results do coincide with those reported by Ohtakara et al. (*25*), whose reports suggest that immobilization of glucoamylase on a chitosan bead through a covalent bond showed a greater stability than immobilization by physical adsorption. Likewise, *Bacillus acidopullulyticus* pullanase immobilized by ionic binding on Amperalite IRC-50 showed less enzyme stability than the enzyme immobilized by covalent binding on chitosan (*26*). Furthermore, Gottschalk and

Jaenicke (*27*) reported that immobilized enzymes by covalent binding (multiple-point attachment) or by entrapment resulted in an increase in the enzyme rigidity, which is commonly reflected by increase in stability toward denaturation.

The calculated values of kinetic parameters K_m (Michaelis constant) and the V_{max} (the maximum reaction rate) for the immobilized enzyme are listed in Table 5. In general, the immobilized enzyme exhibited K_m values higher than that of the free enzyme. This increase is most likely a consequence of either structural changes in the enzyme introduced by the applied immobilization procedure and/or lower accessibility of the substrate to the active site of the immobilized enzyme. The latter, as explained above, may result either from diffusional resistance of the matrix or steric hindrance in the immediate vicinity of the enzyme molecules. Consequently, the maximum rate of the reaction catalyzed by the immobilized enzyme was lower than the free enzyme. Increasing K_m value of hemicelluolase after the immobilization process was similarly reported (*9*).

The Operational Stability of Immobilized Enzymes in Packed-Bead Reactors

The operational stability of the immobilized enzymes was evaluated in continuous operation in packed-bead column-type reactors. The column reactors packed with the immobilized enzymes were prepared for continuous hydrolysis of xylan. The reactors were operated under the optimum conditions of each enzyme as described above. The performance of the reactors is shown in Fig. 1. The activity of the enzymes immobilized on Dowex-50W (ionic binding) and Tannin-chiosan (physical adsorption) was decreased rapidly during the first 10 d, and then little by little over extended periods of time. Thus, after continuous operation for 40 d, the residual activity of the enzyme immobilized on Dowex-50W and tannin-chitosan fell to 18 and 33%, respectively, of the initial conversion rate. The enzymes entrapped in polyacrylamide showed a stable rate for reducing sugar production for 40 d. However, the yield of conversion was very low. On the other hand, the enzymes covalently bounded to chitosan were able to keep producing considerably good amounts of reducing sugars with high stability for 40 d. In addition, the immobilized enzyme retained 81% of its initial conversion rate after 40 d of continuous operation. The rapid drop in the activity (5–7%) during the first period (1–5 d) is possibly owing to the release of untightly bound enzymes from chitosan support. The stability of xylanase and β-xylosidase immobilized on chitosan (covalent binding) is favorably comparable with that reported for other immobilized xylanase and β-xylosidases from *A. niger* (*9,10*).

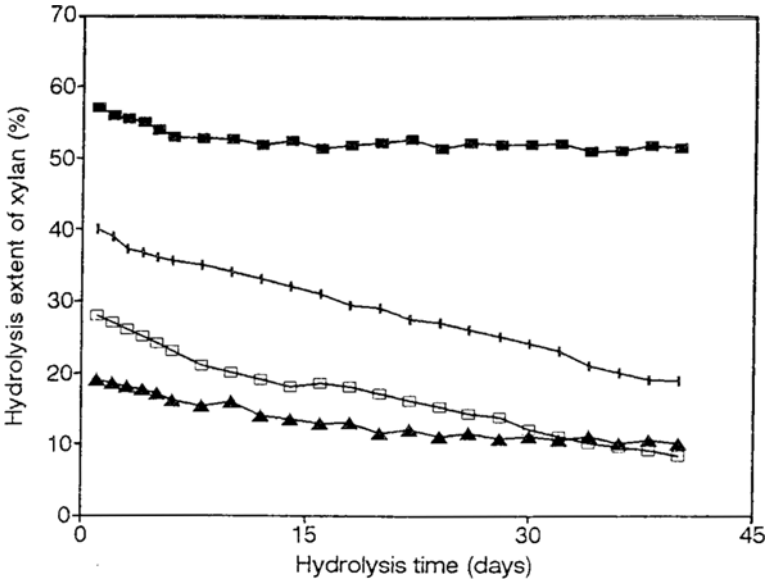

Fig. 1. Operational stability of immobilized enzymes. The reactors were prepared as described in the text. The operation conditions: pH 4.2 and 52.5°C for the enzyme immobilized on Tannin-chitosan (—|—), pH 3.8 and 50°C for the enzyme immobilized on Dowex-50W (—□—), pH 4.0 and 62°C for the enzyme immobilized on chitosan (—■—), pH 4.2 and 65°C for the enzyme entrapped in polyacrylamide (—▲—). The substrate (1% larch wood xylan) was supplied to the reactors at a flow rate of 30 mL/h.

CONCLUSION

In this study, *A. niger* xylanase and β-xylosidase were immobilized on various carriers by different immobilization techniques, viz. Physical adsorption on tannin-chitosan, covalent binding on chitosan, ionic binding on Dowex-50W, and entrapment in polyacrylamide. A considerably good loading efficiency, but low operational stability was exhibited by the physical adsorption on tannin-chitosan, whereas both low loading efficiency and stability were shown by the ionically bound enzymes on Dowex-50W. This was probably because the enzymes are bound to this support through a weak force involving ion exchange, and such bonds are sensitive to the changes of pH and ionic strength. A lower loading efficiency, but better operational stability was exhibited by the enzymes entrapped in polyacrylamide. On the other hand, the enzymes covalently bounded to chitosan through GA showed the highest loading efficiency and operational stability. Furthermore, the immobilization on chitosan

offers various advantages: It is relatively inexpensive and easy to regenerate; the relative surface area, apparent density, and compressive strength of the beads are stable even after autoclaving. It is suggested that chitosan is better used for hemicellulase immobilization.

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