



Lactose hydrolysis by β galactosidase immobilized on concanavalin A-cellulose in batch and continuous mode

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ABSTRACT

β Galactosidase from *Aspergillus oryzae* was immobilized on an inexpensive bioaffinity support, (concanavalin A) Con A-cellulose. The mode of interaction between Con A-cellulose and β galactosidase is shown by Fourier transform infrared spectroscopy. Con A-cellulose adsorbed and crosslinked β galactosidase preparation retained 78% of the initial activity. Soluble and immobilized β galactosidase showed the same pH-optimum at pH 4.6. The temperature-optimum was increased from 50 to 60 °C for the immobilized β galactosidase. The immobilized enzyme had higher thermal stability at 60 °C. The crosslinked adsorbed enzyme retained 80 and 70% of the original enzyme activity in the presence of 3% calcium chloride and 3% galactose, respectively. Moreover, the adsorbed crosslinked and adsorbed β galactosidase exhibited 84 and 75% enzyme activity even after their sixth repeated use, respectively. The crosslinked adsorbed enzyme retained 93% activity after 1 month storage while the native enzyme showed only 63% activity under similar incubation conditions. Immobilized β galactosidase showed higher lactose hydrolysis from solution in batch process at 60 °C as compared to its hydrolysis at 50 °C. The continuous hydrolysis of lactose was appreciably different at various flow rates. Thus, the reactor filled with crosslinked Con A-cellulose adsorbed β galactosidase could be successfully employed for the continuous hydrolysis of lactose from milk and whey.

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1. Introduction

β Galactosidase (3.2.1.23) is a hydrolytic enzyme that catalyzes breakdown of lactose into glucose and galactose. This enzyme is widely present in microbes, plants and animals [1,2]. β Galactosidase from *Aspergillus oryzae* is monomeric, having molecular weight of 105 kDa and a *pI* of 4.6. This enzyme showed pH optima of 4.5 with ONPG and 4.8 with lactose [3–5]. Enzymatic hydrolysis of lactose is a popular technology used to produce lactose-reduced milk and its derived dairy products for consumption by lactose-intolerant persons whose metabolism exhibited a decline in the level of β galactosidase activity [6–8].

The use of soluble enzymes has certain inherent limitations such as stability, reusability and their application in the continuous processes [9,10]. In order to overcome these difficulties, the immobilization of enzymes has been suggested. Immobilization of β galactosidase can be achieved by various methods such as adsorption, covalent attachment, chemical aggregation, entrapment and

micro-encapsulation [2,7,11,12]. Recently, bioaffinity based procedures have attracted the attention of the enzymologist for the immobilization of enzymes. These methods are highly specific for immobilizing enzymes and even the enzymes from partially purified or crude preparations can be immobilized on the support [13,17]. Bioaffinity based supports that have been employed earlier for the immobilization of β galactosidase are quite expensive and time consuming [2,11,14–16].

In this study, an effort has been made to prepare an inexpensive and efficient bioaffinity support, concanavalin A (Con A)-cellulose for the immobilization of *A. oryzae* β galactosidase. Effect of various denaturing agents on the activity of soluble β galactosidase (S β G), Con A-cellulose adsorbed β galactosidase (C β G) and crosslinked Con A-cellulose adsorbed β galactosidase (CC β G) has been investigated. Soluble and crosslinked β galactosidase preparation have been evaluated for the hydrolysis of lactose in batch process at varying temperatures and in continuous reactors at different flow rates.

2. Materials and methods

2.1. Materials

A. oryzae β galactosidase (3.2.1.23) and α -methyl β D-glucopyranoside were obtained from Sigma Chem. Co. (St.

Abbreviations: Con A, concanavalin A; ONPG, *o*-nitrophenyl β -D-galactopyranoside; S β G, soluble β galactosidase; C β G, Con A-cellulose adsorbed β galactosidase; CC β G, crosslinked Con A-cellulose adsorbed β galactosidase.

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Louis, MO, USA). *o*-Nitrophenyl β -D-galactopyranoside (ONPG), ethanolamine and cellulose were obtained from SRL Chemicals (Mumbai, India). Glutaraldehyde was purchased from Thomas Baker Chemical Co. (Mumbai, India). Jack bean meal was procured from Loba Chemicals Co. (Mumbai, India). All other chemicals and reagents employed were of analytical grade and used without any further purification.

2.2. Preparation of Con A

Jack bean meal (10.0 g) was stirred in 100 mL of 0.1 M Tris–HCl buffer, pH 6.1 containing 0.1 M NaCl, 0.001 M MgCl₂, 0.001 M MnCl₂ and 0.001 M CaCl₂ for 2 h at room temperature. Insoluble residue was removed by centrifugation at 12,000 \times g on a cooling centrifuge for 20 min to obtain a clear supernatant. The collected supernatant was used as a source of Con A [14].

2.3. Preparation of bioaffinity support

Cellulose powder (5.0 g) was suspended in 100 mL distilled water and kept overnight for swelling under stirring conditions. The fine particles present in the suspension were removed by decantation and this procedure was repeated at least thrice. The washed cellulose was then suspended in 45 mL of jack bean extract (10%, w/v) and stirred overnight. Con A-cellulose was collected by centrifugation at 10,000 \times g on a cooling centrifuge for 20 min and was washed 2–3 times with 0.1 M sodium phosphate buffer, pH 6.2. The obtained precipitate was used for the immobilization of β galactosidase.

2.4. Biospecific adsorption of β galactosidase on Con A-cellulose support

β Galactosidase (1954 U) was mixed with Con A-cellulose and this mixture was stirred overnight on a magnetic stirrer at 30 °C in sodium acetate buffer, pH 4.6. The precipitate was collected after centrifugation at 10,000 \times g for 20 min. Con A-cellulose adsorbed β galactosidase was washed 2–3 times with 0.1 M sodium acetate buffer, pH 4.6 and finally suspended in the assay buffer and stored at 4 °C for further use [15].

2.5. Crosslinking of Con A-cellulose adsorbed β galactosidase

β Galactosidase adsorbed on Con A-cellulose was crosslinked by 0.5% (v/v) glutaraldehyde for 2 h at 4 °C. Finally this preparation was treated with ethanolamine 0.01% (v/v) for 90 min at 30 °C to stop crosslinking. The integrity of crosslinked Con A-cellulose adsorbed β galactosidase was examined by incubating it in 1.0 M methyl- α -D-glucopyranoside for 2 h.

2.6. Effect of pH

Enzyme activity (2.0 U) was assayed in the buffers of different pH (pH 3.0–9.0). The buffers used were glycine–HCl (pH 3.0), sodium acetate (pH 4.0–4.5), sodium phosphate (5.0–7.0) and Tris–HCl (pH 8.0–9.0). The molarity of each buffer was 0.1 M. The activity at pH 4.6 was taken as control (100%) for the calculation of remaining percent activity.

2.7. Effect of temperature

The effect of temperature on soluble and immobilized β galactosidase (2.0 U) was studied by measuring the activity of the enzyme preparations at various temperatures (20–80 °C).

The stability of the soluble and immobilized enzyme preparations was investigated by incubating them at 60 °C in 0.1 M sodium

acetate buffer, pH 4.6 for various times. Aliquots from each preparation (2.0 U) were collected at indicated times and kept in ice for 10 min. After incubation in ice, the enzyme was brought at room temperature. The enzyme activity without incubation at 60 °C was taken as control (100%) for the calculation of remaining percent activity.

2.8. Effect of calcium chloride

β Galactosidase (2.0 U) was incubated with increasing concentrations of CaCl₂ (1.0–5.0%, w/v) in 0.1 M sodium acetate buffer, pH 4.6 for 1 h and its activity was determined by measuring the release of *o*-nitrophenol from ONPG at 405 nm. The activity of enzyme without added calcium chloride was considered as control (100%) for the calculation of remaining percent activity.

2.9. Effect of galactose

The effect of various concentrations of galactose (1.0–5.0%, w/v) on the activity of soluble and immobilized β galactosidase (2.0 U) was measured in 0.1 M sodium acetate buffer, pH 4.6 at 37 °C. The activity of enzyme without added galactose was considered as control (100%) for the calculation of remaining percent activity.

2.10. Storage stability

Soluble and crosslinked adsorbed β galactosidase was stored at 4 °C in 0.1 M sodium acetate buffer, pH 4.6 for 2 months. The aliquots from each preparation (2.0 U) were taken in triplicates at the gap of 10 days and were then analyzed for the remaining activity. The activity determined on the first day was taken as control (100%) for the calculation of remaining percent activity [16].

2.11. Reusability of immobilized β galactosidase

Immobilized β galactosidase preparations (2.0 U) were taken in triplicates for assaying the activity of enzyme. After each assay, immobilized enzyme was taken from assay tubes and stored in 0.1 M sodium acetate buffer, pH 4.6 overnight at 4 °C for 6 successive days. The activity determined on the first day was considered as control (100%) for the calculation of remaining percent activity.

2.12. FTIR spectra of Con A-cellulose and Con A-cellulose adsorbed β galactosidase

FTIR spectra of Con A-cellulose and Con A-cellulose adsorbed β galactosidase were monitored with INTERSPEC 2020 model FTIR instrument, USA. The calibration was done by polystyrene film. The samples were injected by Hamiet 100 μ L syringe in ATR box. The syringe was first washed by acetone followed by distilled water. FTIR analysis was done to monitor the functional groups of the compounds.

2.13. Lactose hydrolysis in batch process

Lactose solution (500 mL, 0.1 M) was independently incubated with soluble and crosslinked adsorbed β galactosidase (400 U) and stirred continuously in water bath at 50 and 60 °C for varying times. The aliquots were taken at different times and assayed for the formation of glucose by using glucose oxidase–peroxidase coupled assay procedure [14].

2.14. Hydrolysis of lactose in the continuous reactors filled with immobilized enzyme

Three different reactors of similar dimensions (2.0 cm × 10.0 cm) were independently packed with crosslinked Con A-cellulose adsorbed β galactosidase. The packed-bed volume of the column was 8.0 mL. Lactose (0.1 M) dissolved in 0.1 M sodium acetate buffer, pH 4.6 containing 0.001 M sodium azide was passed through all the three reactors at different flow rates (10, 20 and 30 mL h⁻¹) at room temperature (30–32 °C).

2.15. Glucose estimation by glucose oxidase–peroxidase coupled assay procedure

The lactose hydrolysis was monitored for the formation of glucose by using glucose oxidase–peroxidase coupled assay procedure. An appropriate amount of lactose hydrolyzed by β galactosidase, suitably diluted with 0.5 M phosphate buffer, pH 6.1 was taken. The hydrolysis of lactose was estimated by using solution C. Solution C was prepared by taking 5.0 mg glucose oxidase, 15.0 mg o-dianisidine HCl prepared in 2.5 mL of distilled water, 40.0 mL glycerol (20%, v/v) and 1.0 mg peroxidase dissolved in 5.0 mL of 0.1 M potassium phosphate buffer, pH 6.1. In each tube, 1.5 mL of solution C was added. The test tubes were again incubated at 37 °C for 15 min and the reaction was stopped by adding 1.0 mL of 6.0N HCl and the developed colour was measured at 540 nm [14].

2.16. Assay of β galactosidase

The ONPG hydrolyzing activity of β galactosidase was determined by measuring the release of o-nitrophenol at 405 nm [13]. The reaction was carried out with continuous shaking in an assay volume of 2.0 mL containing 1.7 mL of 0.1 M sodium acetate buffer, pH 4.6, 0.1 mL β galactosidase (2.0 U) and 0.2 mL of 20 mM ONPG. The reaction was stopped by adding 2.0 mL of 1.0 M sodium carbonate solution and o-nitrophenol formation was measured spectrophotometrically at 405 nm.

One unit (1.0 U) of β galactosidase activity is defined as the amount of enzyme that liberates 1.0 μmol of o-nitrophenol (ε_m = 4500 L mol⁻¹ cm⁻¹) per min under standard assay conditions.

2.17. Estimation of protein

Protein concentration was determined according to the procedure described by Lowry et al. [18]. Bovine serum albumin was used as a standard.

3. Results

3.1. Immobilization of β galactosidase on bioaffinity support

Table 1 demonstrates the immobilization of β galactosidase on Con A-cellulose support. Con A-cellulose adsorbed β galactosidase retained nearly 82% of the original activity. However, the crosslinking of the adsorbed enzyme with glutaraldehyde resulted in a marginal loss of 4% enzyme activity.

Table 1 β Galactosidase immobilized on Con A-cellulose.

Enzyme preparation	Enzyme activity loaded, X (U)	Enzyme activity in washes, Y (U)	Activity bound g ⁻¹ Con A-cellulose		Activity yield (%), B/A × 100
			Theoretical (X - Y) = A	Actual = B	
Enzyme adsorbed on Con A-cellulose	1954	409	1545	1269	82
Enzyme adsorbed on Con A-cellulose and crosslinked	1269	0	1269	1220	78

Each value represents the mean for three independent experiments performed in duplicates, with average standard deviations, <5%.

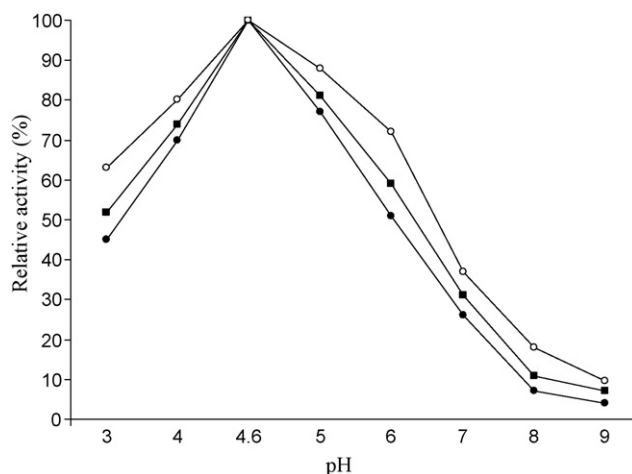


Fig. 1. pH-activity profiles for soluble and immobilized β galactosidase. The activity of soluble and immobilized β galactosidase (2.0 U) preparations was measured in the buffers of various pH (3.0–9.0). The buffers used were glycine–HCl (pH 3.0), sodium acetate (pH 4.0–4.5), sodium phosphate (5.0–7.0) and Tris–HCl (pH 8.0–9.0). Molarity of each buffer was 0.1 M. The activity at pH 4.6 was taken as control (100%) for the calculation of remaining percent activity. The symbols show SβG (●) and CβG (○).

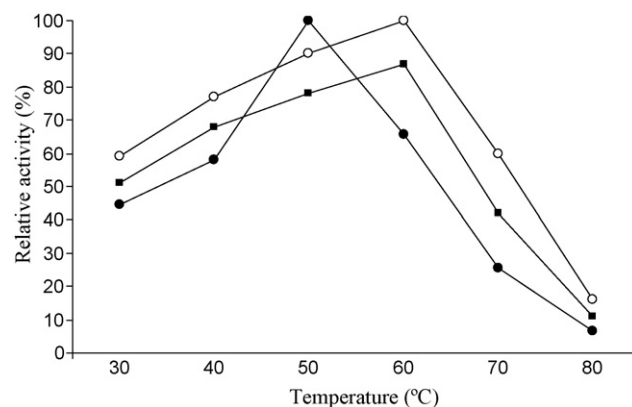


Fig. 2. Temperature activity profiles for soluble and immobilized β galactosidase. The activity of soluble and immobilized β galactosidase (2.0 U) preparations was assayed in 0.1 M sodium acetate buffer, pH 4.6 at various temperatures (20–80 °C) for 15 min. The activity obtained at 50 °C was considered as control (100%) for the calculation of remaining percent activity for soluble enzyme and the activity obtained at 60 °C was considered as control (100%) for the calculation of remaining percent activity of the immobilized enzyme. For symbols, please refer to Fig. 1 legend.

3.2. Effect of pH and temperature

Fig. 1 demonstrates the pH-activity profiles for soluble and immobilized β galactosidase preparations. The soluble and immobilized β galactosidase showed same pH-optimum, pH 4.6.

The temperature-optimum was increased from 50 °C for free enzyme to 60 °C for the immobilized enzyme preparations (Fig. 2). However, crosslinked adsorbed β galactosidase retained higher

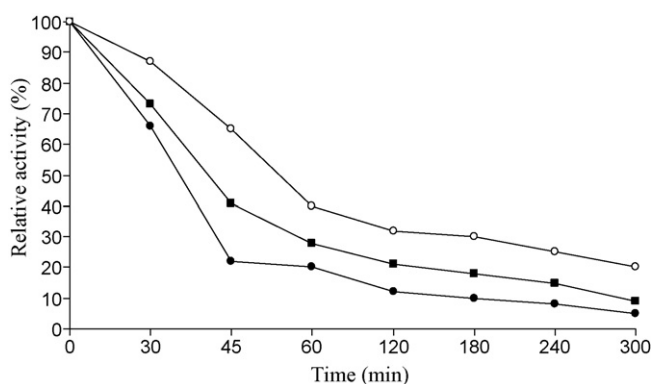


Fig. 3. Thermal denaturation for soluble and immobilized β galactosidase. Soluble and immobilized β galactosidase preparations were incubated at 60 °C in 0.1 M sodium acetate buffer, pH 4.6 for varying times. Aliquots of each preparation (2.0 U) were taken out at indicated time intervals and chilled quickly in crushed ice for 5 min. The activity of enzyme obtained without incubation at 60 °C was taken as control (100%) for the calculation of remaining percent activity. For symbols, please refer to Fig. 1 legend.

fractions of catalytic activity at other temperatures as compared to simply adsorbed β galactosidase preparation.

Fig. 3 illustrates the thermal inactivation of soluble and immobilized β galactosidase. The crosslinked Con A-cellulose bound enzyme retained 65% of the initial activity after 45 min exposure at 60 °C while the soluble enzyme exhibited marginal activity of 22% under similar exposure. Further incubation of soluble β galactosidase at 60 °C for 5 h resulted in a loss of almost 97% activity whereas crosslinked Con A-cellulose adsorbed β galactosidase retained more than 20% activity.

3.3. Effect of calcium chloride

The activity of soluble and immobilized β galactosidase was evaluated in the presence of different concentrations of CaCl_2 (Fig. 4). The pre-incubation of soluble β galactosidase with 2.0% and 5.0% CaCl_2 for 1 h at 37 °C resulted in the loss of 32 and 60% activity, respectively, whereas crosslinked adsorbed β galactosidase retained significantly very high activity 80% and 60% under identical exposure.

3.4. Effect of galactose

Galactose is one of the products of β galactosidase catalyzed hydrolysis of lactose. The activity of soluble and immobilized β

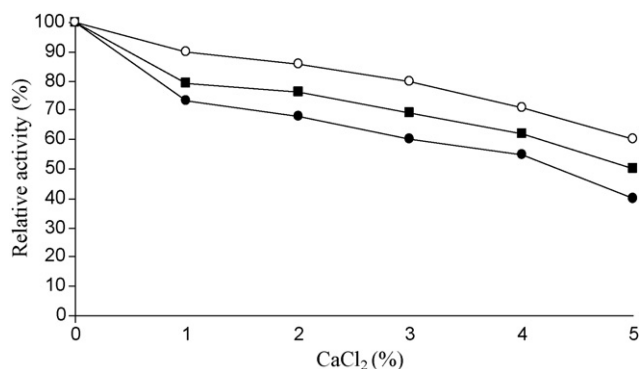


Fig. 4. Effect of calcium chloride on soluble and immobilized β galactosidase. Soluble and immobilized β galactosidase (2.0 U) preparations were incubated with increasing concentrations of CaCl_2 (1.0–5.0%, w/v) in 0.1 M sodium acetate buffer, pH 4.6 for 1 h at 37 °C. The activity of the enzyme was determined as described in the text. For symbols, please refer to Fig. 1 legend.

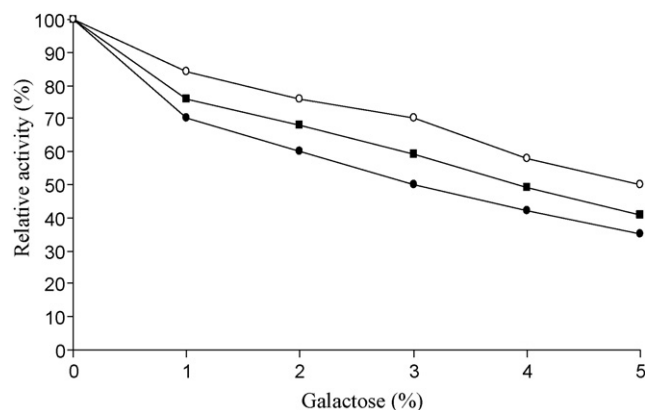


Fig. 5. Effect of galactose on soluble and immobilized β galactosidase. Effect of galactose on soluble and immobilized β galactosidase (2.0 U) preparations was measured in the presence of increasing concentrations of galactose (1.0–5.0%, w/v) in 0.1 M sodium acetate buffer, pH 4.6 for 1 h at 37 °C. The activity of enzyme without galactose was considered as control (100%) for the calculation of remaining percent activity at various concentrations of galactose. For symbols, please refer to Fig. 1 legend.

galactosidase was investigated in the presence of various concentrations of galactose (Fig. 5). The incubation of soluble β galactosidase with 5.0% galactose for 1 h at 37 °C showed only 35% activity while crosslinked Con A-cellulose adsorbed β galactosidase retained over 50% activity under similar incubation conditions.

3.5. Storage stability

Table 2 depicts the storage stability for soluble and crosslinked Con A-cellulose bound β galactosidase. The crosslinked adsorbed enzyme retained 79% of the initial enzyme activity after 2 months of storage while its soluble counterpart exhibited only 43% activity under similar storage conditions.

3.6. Reusability of immobilized beta galactosidase preparations

The reusability of the immobilized β galactosidase preparations has been shown in Fig. 6. Both Con A-cellulose adsorbed β galactosidase and crosslinked adsorbed enzyme preparations showed 75 and 84% of the initial activity after their 6th repeated use, respectively.

3.7. FTIR analysis

FTIR spectra for Con A-cellulose and crosslinked Con A-cellulose adsorbed β galactosidase were recorded in the range of 1000–4000 cm^{-1} (Fig. 7). The IR spectrum for Con A-cellulose

Table 2
Storage stability of soluble and immobilized β galactosidase at 4 °C.

Number of days	Remaining activity (%)	
	SβG	CCβG
Control	100	100
10	94	98
20	75	95
30	63	93
40	55	89
50	48	85
60	43	79

SβG and CCβG preparations were stored at 4 °C in 0.1 M sodium acetate buffer, pH 4.6 for over 2 months. The aliquots from each preparation (2.0 U) were taken in triplicates at the gap of 10 days and were then analyzed for the remaining enzyme activity. Each value represents the mean for three independent experiments performed in duplicates, with average standard deviations, <5%.

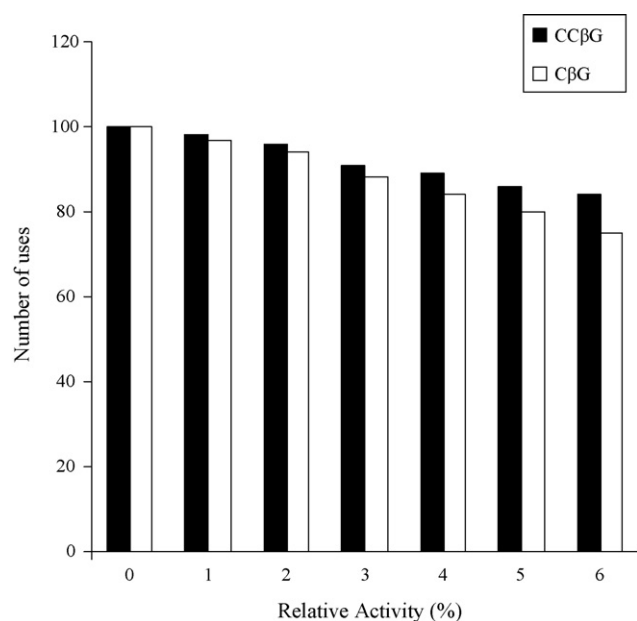


Fig. 6. Reusability of immobilized β galactosidase. The reusability of adsorbed β galactosidase and crosslinked adsorbed β galactosidase was monitored for 6 successive days. The aliquots from both preparations were taken in triplicates and were assayed for the remaining percent activity. The activity determined on the first day was taken as control (100%) for the calculation of remaining activity after each use.

Table 3

Hydrolysis of lactose by soluble and immobilized β galactosidase in batch process at different temperatures.

Time (h)	Temperature ($^{\circ}$ C)			
	Remaining activity (%)			
	50 $^{\circ}$ C		60 $^{\circ}$ C	
	SβG	CCβG	SβG	CCβG
Control	0	0	0	0
1	69	52	64	55
2	74	63	64	58
3	77	69	67	64
4	77	76	70	69
5	78	79	74	84
6	80	84	74	90
7	80	84	74	90
8	80	86	74	90
9	82	86	74	90
10	82	86	74	90

Lactose hydrolysis was performed as described in text (Section 2.12). Each value represents the mean for three independent experiments performed in duplicates, with average standard deviations, <5%.

showed the presence of NH_2 group at 1643.64 cm^{-1} while the presence of $\text{C}=\text{O}$ group in Con A-cellulose was confirmed by the peak value at 1053.28 cm^{-1} .

3.8. Lactose hydrolysis in batch process

Table 3 illustrates the hydrolysis of lactose by soluble and crosslinked Con A-cellulose adsorbed β galactosidase for 10 h at 50 and 60 $^{\circ}$ C. It was observed that the hydrolysis of lactose by soluble β galactosidase was 77% after 3 h at 50 $^{\circ}$ C whereas the crosslinked adsorbed enzyme exhibited 69% of the hydrolytic activity under similar conditions. The soluble β galactosidase hydrolyzed 82% lactose after 9 h while the crosslinked adsorbed enzyme hydrolyzed greater amount of lactose 86% in only 8 h.

The hydrolysis of lactose at 60 $^{\circ}$ C after 1 h was 64 and 55% by the free and crosslinked adsorbed β galactosidase, respectively. It was noticed that 74% of lactose was hydrolyzed after 5 h by the free enzyme. However, the hydrolysis of lactose by soluble enzyme beyond this limit does not exhibit any significant increase whereas the lactose hydrolysis by crosslinked Con A-cellulose adsorbed β galactosidase was reached to 90% in 6 h (Table 3).

3.9. Hydrolysis of lactose through the packed-bed reactors filled with crosslinked Con A-cellulose adsorbed β galactosidase

The rate of lactose hydrolysis was monitored at different flow rates in various reactors containing crosslinked Con A-cellulose adsorbed β galactosidase. The greater percent of lactose was hydrolyzed inside the column when the flow rate was 10 mL h^{-1} while the hydrolytic rates slightly decreased as the flow rate was increased to 20 and 30 mL h^{-1} . It was seen that 95% lactose was hydrolyzed by the immobilized β galactosidase present in the reactor after 10 days of continuous operation when the flow rate was 10 mL h^{-1} . Lactose hydrolysis was 90% after 2 months of the continuous operation of the reactor at a flow rate of 10 mL h^{-1} . Moreover, lactose was hydrolyzed to 87 and 80% at the flow rate of 20 and 30 mL h^{-1} at the end of 2 months of continuous operation of the reactor, respectively (Table 4).

4. Discussion

The present study deals with the immobilization of β galactosidase on an inexpensive Con A-cellulose, a bioaffinity support. Con A-cellulose was prepared by using jack bean extract [14,18,19]

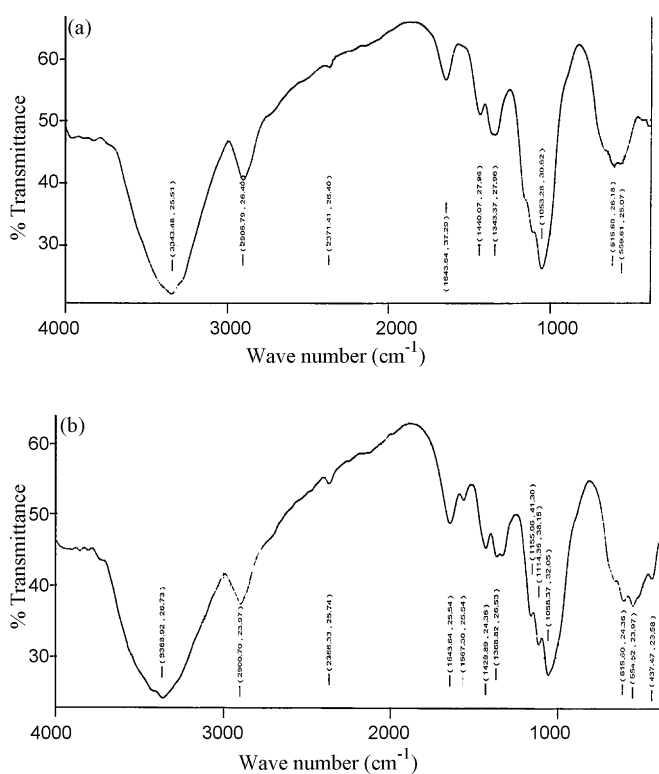


Fig. 7. FTIR spectra of Con A-cellulose and crosslinked Con A-cellulose adsorbed β galactosidase. FTIR analysis was done to monitor the linkage between Con A-cellulose and β galactosidase. The FTIR spectra for Con A-cellulose and crosslinked Con A-cellulose adsorbed β galactosidase have been shown in (a) and (b), respectively.

Table 4
Lactose hydrolysis in the columns filled with CCβG at different flow rates.

Number of days	Lactose hydrolysis (%) Flow rate (mL h ⁻¹)		
	10	20	30
Control	100	100	100
5	95.0	91.45	87.3
10	95.0	91.45	87.0
15	94.3	90.0	87.0
20	94.3	90.0	87.0
25	94.3	89.8	84.9
30	92.0	89.8	84.9
35	92.0	89.8	83.0
40	92.0	88.4	83.0
45	91.8	87.0	81.7
50	90.0	87.0	81.7
55	90.0	87.0	80.0
60	90.0	86.9	80.0

Hydrolysis of lactose in the continuous reactors filled with CCβG (2000 U) was performed as described in text (Section 2.13). Each value represents the mean for three independent experiments performed in duplicates, with average standard deviations, <5%.

and this bioaffinity support was exploited for the surface immobilization of β galactosidase. Several investigators have earlier reported the use of glutaraldehyde as crosslinking agent in different ways [20–22]. Here, Con A-cellulose adsorbed β galactosidase was crosslinked by glutaraldehyde in order to prevent the dissociation of enzyme from the support. Crosslinking of enzyme resulted in a marginal loss of enzyme activity (Table 1). Several workers have already reported that crosslinking of adsorbed enzymes by glutaraldehyde prevented the dissociation of enzymes from Con A supports in the presence of glucose, mannose and N-acetyl glucosamines and it also significantly enhanced stability of enzymes against various physical and chemical denaturants [18,19,23].

There was a shift in the temperature-optimum for the immobilized β galactosidase from 50 to 60 °C (Fig. 2). El-Masry et al. [24] previously showed that β galactosidase from *A. oryzae* immobilized via diazotization or condensation on nylon membranes grafted with glycidyl methacrylate had a higher temperature-optimum than its soluble counterpart. This increased activity at higher temperatures showed significant stabilization against heat induced inactivation. Crosslinked Con A-cellulose adsorbed β galactosidase has shown remarkably very high stabilization against heat induced denaturation (Fig. 3). Tanriseven and Dogan [25] also reported similar findings when the enzyme was immobilized in fibers composed of alginate and gelatin hardened by glutaraldehyde. Moreover, crosslinking of enzyme with glutaraldehyde, a bi-functional agent enhanced thermal stability of the enzyme due to the formation of several linkages between enzyme and support [10,23].

Milk is a common health drink consumed by people of all age groups. Calcium is one of the major minerals found in milk that is required in large quantities for bone growth, development of soft tissues and to maintain the casein micelle structure. The deficiency of calcium can thus lead to several disorders in an individual [8,26]. Therefore, we have investigated the effect of CaCl₂ on the activity of soluble and immobilized β galactosidase. Immobilized enzyme exhibited significantly higher stabilization against exposure to Ca²⁺ ions (Fig. 4). Haider and Husain [19] earlier reported that the soluble enzyme exposed to 5% CaCl₂ for 1 h at 37 °C resulted in a loss of nearly half of the original activity while the calcium alginate entrapped crosslinked Con A-β galactosidase retained more than 80% of the original activity.

Galactose is one of the products of β galactosidase catalyzed hydrolysis of lactose. Our findings indicated that the crosslinked adsorbed Con A-cellulose β galactosidase and adsorbed Con A-cellulose β galactosidase were significantly more resistant to the

inhibition mediated by galactose as compared to the native enzyme (Fig. 5). Some earlier investigators have also reported the competitive inhibition of β galactosidase by its reaction product, galactose [27–29].

FTIR spectra reveal the presence of amino group at 1643.64 cm⁻¹ which was due to free -NH₂ groups of Con A [30]. The presence of C=O group in β galactosidase immobilized on Con A-cellulose has been confirmed by its peak value at 1053.28 cm⁻¹. Moreover, the peak at 1343.37 and 1440.07 cm⁻¹ further confirmed the presence of -C-N groups in this complex (Fig. 7). We further state that major shift of peak occurs within the region of 1000–2000 cm⁻¹ due to binding of Con A-cellulose with β galactosidase. It was also observed visually by change in colour from white to pale yellow. This interaction evidenced the formation of strong poly-electrolyte complex [31].

The results for the continuous hydrolysis of lactose in batch process exhibited that the rate of hydrolysis was more in case of soluble β galactosidase for the first few hours as compared to crosslinked Con A-cellulose adsorbed β galactosidase (Table 3). This was due to the fact that the soluble enzyme was more accessible for the hydrolysis of lactose for first few hours but after prolonged incubation, the rate of lactose hydrolysis decreased because of more inhibition of soluble enzyme by its own product. This phenomenon of inhibition of soluble β galactosidase by its products has already been explained earlier [32]. Immobilized β galactosidase was found to be more resistant to inhibition by product thus it hydrolyzed more lactose on prolong incubation. Pessela et al. [33] have also reported more hydrolysis of lactose by immobilized enzyme as compared to soluble β galactosidase.

Lactose hydrolysis was also analyzed at different flow rates by the crosslinked Con A-cellulose bound β galactosidase. The greater extent of lactose was hydrolyzed by the enzyme present in the column at the flow rate of 10 mL h⁻¹ (Table 4). It was due to more residence time of lactose inside the column. A similar finding for the continuous hydrolysis of lactose in a continuous column by calcium alginate entrapped β galactosidase has been reported by Haider and Husain [14]. The greater stability of this inexpensive immobilized β galactosidase preparation at higher temperatures for longer duration might bring about its use in the continuous hydrolysis of lactose at large scale.

5. Conclusion

This study deals with a simple and inexpensive procedure for the immobilization of β galactosidase by using inexpensive Con A and cellulose. Immobilized β galactosidase exhibited significantly higher stability against various types of denaturants and on storage than the free enzyme. The reusability experiment demonstrated that the enzyme does not dissociate from the matrix appreciably upon repeated uses and thus this immobilized β galactosidase preparation could be successfully employed for the hydrolysis of lactose from milk and whey in a batch process. In view of the stability and utility of immobilized enzyme to hydrolyze lactose in batch and continuous processes, such preparation could be exploited for the continuous hydrolysis of lactose from milk and whey continuously for longer durations in reactors.

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