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Immobilization of soybean (*Glycine max*) α-amylase onto Chitosan and Amberlite MB-150 beads: Optimization and characterization

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ABSTRACT

α-Amylase from soybeans was immobilized on two different matrices, Chitosan beads and Amberlite MB-150. Maximum immobilization of 62% and 70.4% was obtained with Chitosan and Amberlite MB-150, respectively. The optimum pH obtained was 8.0 and 7.0 for the α-amylase immobilized on Chitosan beads and Amberlite MB-150, respectively; free enzyme showed an optimum pH of 5.5. The optimum temperature for both free and Chitosan immobilized enzymes was 70 °C whereas it was 75 °C for enzyme immobilized on Amberlite MB-150. α-Amylase immobilized on Chitosan showed an apparent K_m of 4 mg/mL, whereas Amberlite immobilized enzyme showed an apparent K_m of 2.5 mg/mL. The immobilized enzyme showed a high operational stability by retaining 38% and 58% of initial activity after 10 uses for Chitosan and Amberlite, respectively. The easy accessibility of soybean α-amylase, the ease of its immobilization on low-cost matrices, increased stability upon immobilization make it a suitable product for future applications. Both the matrices used for enzyme immobilization are non-toxic, cheap, renewable, biodegradable and have importance in food, cosmetics, biomedical, or pharmaceuticals applications.

1. Introduction

Enzymes are biocatalyst which accelerate the chemical reactions of living cells. Enzymes are highly effective and specific under ambient conditions; enzymatic hydrolysis allows greater control over amylolysis, the specificity of the reaction, and the stability of generated products [1,2]. Recovery of enzymes from reaction solutions and separation of the enzymes from substrates and products are in general very difficult. This problem can be resolved by immobilization of the enzyme on various matrices.

Enzyme immobilization refers to the preparation of water-insoluble biocatalytic derivatives and involves the coupling of enzymes to solid supports which often possess improved storage and operational stability. Enzymes can be covalently bonded via various chemical bonding methods, such as cross-linking, multifunctional reagents, or surface reactive functional groups [3–10]. Inorganic carriers, such as silica [11], glass beads [12], and corn grits [13] are also used for immobilization of enzyme. Recently, different methods and advance approaches of enzyme immobilization have been well-reviewed [14,15].

α-Amylase catalyzes the hydrolysis of α-(1 → 4) glucosidic linkages of polysaccharides such as starch, glycogen, or their degradation products [16–19]. The hydrolysis of starch to low molecular weight sugars by α-amylases is one of the most important commercial enzymatic processes [20–22]. Their application in food and starch based industries is the major market, and further the demand for α-amylases would always be evergreen in these sectors [23,24]. There have been many reports about immobilization of α-amylase on various matrices [25–44]. Covalent binding is very effective in retaining the enzyme activity and provides a maximum rigidity and also prevents enzyme from unfolding upon heating or in the presence of a denaturant.

Chitosan is a polysaccharide made up of 2-amino-2-deoxy-D-glucose units, which are joined by β-1,4-linkages and is obtained by deacetylation with drastic alkaline treatment of chitin [45]. In linear polyglucosamine chains, Chitosan has reactive amino and hydroxyl groups, which make possible the coupling of enzymes [46]. Chitin the second most abundant naturally occurring biopolymer after cellulose, is the major structural component of the invertebrate exoskeleton and the fungal cell wall [47]. Depending on the source and preparation, molecular mass may range from 300 to over 1000 kDa.

The degree of deacetylation (% DD) can be determined by NMR spectroscopy, and the % DD in commercial Chitosan is in the range 60–100%. The degradation rate of Chitosan is inversely related to the degree of crystallinity, and thus on deacetylation [48,49]. One of the properties of Chitosan is that it can be molded in various forms [50]. The amino group in Chitosan has a pK_a value of

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~6.5, this polysaccharide becomes water-soluble under acidic conditions (pH <6), allowing the preparation of biocompatible and often biodegradable polymer solutions [51–57]. The advantages are accessibility, cheapness, hydrophilic character and a great number of free amino groups on the surface, capable of chemical reaction [58]. This biocompatible, antibacterial and environmentally friendly polyelectrolyte is used in water treatment, chromatography, additives for cosmetics, and textile treatment for antimicrobial activity [59,60]. They are also used as photographic papers, and biodegradable films [61]. They have many applications in biomedical devices and microcapsule implants for controlled release in drug delivery [62–64].

The cationic nature of Chitosan is mainly responsible for electrostatic interactions with anionic glycosaminoglycans and other negatively charged molecules. Glutaraldehyde is used as cross-linker to bind the free amino group of Chitosan with the enzyme molecule. However, Chitosan as a carrier has comparatively low mechanical resistance. Chitosan has been previously used for immobilization of some plant and bacterial enzymes and DNA [65,9,66–71]. Moreover, the ability of Chitosan to link with anionic glycosaminoglycans, DNA and other negatively charged molecules makes this material a good candidate for industrial uses, gene therapy and in orthopedics.

Amberlite MB-150 is a mixture of strongly acidic cationic and strongly basic anionic resin. Amberlite is resistant to biological degradation and compatible with almost all organic solvents and most concentrated acids. Amberlite MB-150 beads are spherical in moist, fully hydrated condition. Its cation to anion equivalent ratio is 1:1. Its volumetric composition is made of 40% cation and 60% anion resin. Amberlite MB-150 is insoluble in water and dilute solution of acids or bases. It can withstand pH range from 0 to 14 and can be regenerated. Amberlite has been used as a matrix for immobilization for various enzymes such as urease [72,73], lipase [74], and β-galactosidase [75,76]. Furthermore, enzyme immobilization was done using glutaraldehyde as it can bind lysine amino groups of enzyme and cross-link the enzyme with the matrix.

In the present study α-amylase isolated from soybean seeds has been immobilized on Chitosan and Amberlite MB-150 beads. The main advantages of immobilizing soybean α-amylase enzymes are its easy and wide availability, and a simple purification protocol. Physicochemical properties of immobilized enzymes are compared with respect to soluble enzyme. Lastly, the storage stability and reusability of the immobilized enzyme were studied w.r.t. the soluble enzyme.

2. Materials and methods

2.1. Enzymes and chemicals

Soybean α-amylase was purified according to Kumari et al. [77]. Crab shell Chitosan, Amberlite MB-150, DNS, glutaraldehyde were obtained from Sigma Chemical Co. (St. Louis, MO, USA), sodium acetate was procured from Sisco Research Labs, Mumbai. All other chemicals were of analytical grade. All solutions were prepared in Milli Q (Millipore, Bedford, MA, USA) water with a resistance of higher than 18 MΩ cm.

2.2. Immobilization of soybean α-amylase on Chitosan bead

2.2.1. General properties of Chitosan

Chitosan from crab shell used for immobilization was of practical grade. % deacetylation degree was found to be ≥85; viscosity was >200 mPa s.

2.2.2. Preparation of Chitosan beads

Chitosan beads (1.5%) were prepared in 1.5% acetic acid by heating at 45 °C, while constantly stirring the solution [78,79]. This solution was taken in a syringe with nozzle of diameter 5 mm and allowed to fall drop by drop in 100 mL of 1 M KOH solution. Obtained beads were continuously stirred for 2 h at room temperature for hardening of Chitosan beads. Beads of uniform shape and size (diameter 3 mm) were obtained and filtered using Whatman No. 1 filter paper.

2.2.3. Activation of prepared Chitosan beads

Prepared Chitosan beads were activated using glutaraldehyde ranging from 1 to 3% (v/v) and allowed to stand at room temperature for 3 h. Activated Chitosan beads were washed thoroughly with 50 mM sodium acetate buffer pH 5.5 (standard buffer); two or three times to remove any free glutaraldehyde. Activated Chitosan beads were stored at their optimal pH, 50 mM Tris buffer at 4 °C, until used.

2.2.4. Immobilization of soybean α-amylase on activated Chitosan bead

The activated Chitosan beads were incubated with varying amounts of soybean α-amylase protein at different time intervals to obtain maximum immobilization. Beads were washed 4–5 times with standard buffer to remove unbound protein. Chitosan beads were stored at their optimal pH, 50 mM Tris buffer 4 °C, until used.

2.3. Immobilization of soybean α-amylase onto Amberlite MB-150 beads

Method by Anita and Sastry [72] was adopted for the immobilization of soybean α-amylase onto Amberlite MB-150. Amberlite MB-150 beads (50–200 mg) of diameter 5 μm were equilibrated at different pHs ranging from 4 to 8 and activated using 1–3% (v/v) solution of glutaraldehyde for 2 h at room temperature. The glutaraldehyde-activated Amberlite beads were washed with standard buffer two or three times to remove excess glutaraldehyde. Activated Amberlite was treated with different concentrations of soybean α-amylase protein and incubated for different time intervals to obtain maximum immobilization. Matrix containing the Amberlite-amylase was washed with the standard buffer to remove any unbound enzyme. The protein and enzyme activity were estimated in washed off fractions. To determine the optimum immobilization conditions, the following parameters during the immobilization process were studied: (a) Amberlite amount, 50–200 mg, (b) pH, 4.0–8.0, (c) glutaraldehyde concentration, 1–3% (v/v) and (d) protein amount, 3–6 mg. Amberlite-amylase was finally stored in semi-dry condition, without any buffer at 4 °C.

2.4. Enzyme and protein assays

α-Amylase activity assay was carried out by DNS method [80], for both soluble and immobilized enzymes. Two beads were taken for routine assay of the activity of immobilized enzyme on Chitosan. Beads were removed after 10 min incubation with 1 mL starch (1%) and 1 mL DNS was added for color development. The tube containing this reaction mixture was incubated in a boiling water bath for 5 min and then cooled in running tap water. After addition of 10 mL of Milli Q water, absorbance was recorded at 540 nm. For Amberlite immobilized enzyme, matrix was incubated with 1 mL starch (1%) for 10 min and the supernatant was transferred to another test tube. DNS was added for color development and absorbance was recorded at 540 nm. One unit of activity was defined as the amount of enzyme required to produce 1 μmol of reducing sugar/min.

The protein concentration was determined by method of Bradford with crystalline BSA as the standard [81]. The amount of

protein immobilized was estimated by subtracting the amount of protein determined in supernatant after immobilization from the total amount of protein used for immobilization.

2.5. Immobilization efficiency

The efficiency of immobilization onto Chitosan and Amberlite MB-150 was calculated using following formula:

$$\%\ \text{immobilization} = \frac{\text{specific activity of immobilized enzyme}}{\text{specific activity of soluble enzyme}} \times 100$$

where specific activity of immobilized enzyme = specific activity of soluble enzyme − specific activity of unbound enzyme.

2.6. Steady state kinetics

2.6.1. pH

The pH optimum for the soybean α-amylase activity for Chitosan and Amberlite MB-150 was determined by assaying at different pH values using different buffers viz., 50 mM sodium acetate buffer (pH range 3.0–5.6), 50 mM phosphate buffer (pH range 5.7–8.0), 50 mM Tris buffer (pH range 8.0–10.0). The substrate 1% starch was prepared in respective pH buffers and % maximum enzyme activity was calculated. The pH of the immobilized enzyme was compared with the soluble enzyme.

2.6.2. Temperature

The optimum temperature for soluble and immobilized Chitosan and Amberlite MB-150 immobilized enzyme was determined by assaying the enzyme at temperature from 25 °C to 85 ± 1 °C in standard buffer and 50 mM Tris buffer, respectively; in a water bath (Multitemp, Pharmacia, Sweden) at their optimal pH. % maximum activity was calculated as stated above.

2.6.3. K_m and V_{max}

Effect of substrate concentration on amylase activity was investigated at 37 °C by varying the starch concentration from 0 to 10 mg/mL in 50 mM Tris buffer at optimum pH for immobilized enzymes. The activity assay was performed as stated above. Michaelis constant (K_m) and V_{max} was determined using Lineweaver–Burk plot with the SigmaPlot 9.0 software. All parameters were the mean of triplicate determinations from three independent preparations.

2.7. Storage stability

For storage stability studies, immobilized amylase on Chitosan and Amberlite MB-150 beads were kept in 50 mM Tris buffer. Both the beads were stored at 4 °C at their optimal pH. The activity of immobilized enzyme was determined using the assay procedure mentioned in the previous section. Immobilized amylase was tested for % initial activity at regular intervals; similarly the activity of fresh soluble amylase activity was also checked at regular intervals. Freshly immobilized enzyme was taken as control for each assay. After each assay, Chitosan beads and Amberlite were washed with 50 mM Tris buffer and stored at 4 °C as stated above and % initial activity was plotted as a function of time.

2.8. Reusability

The immobilized soybean was reused 10 times over a period of 10 days, and the % initial activity was measured. After the amylase assay, the immobilized Chitosan beads and Amberlite MB-150 were washed with 50 mM Tris buffer (at their optimal pH), dried, and stored at 4 °C. Furthermore, the immobilized amylase, which showed better stability, was reused for prolonged periods.

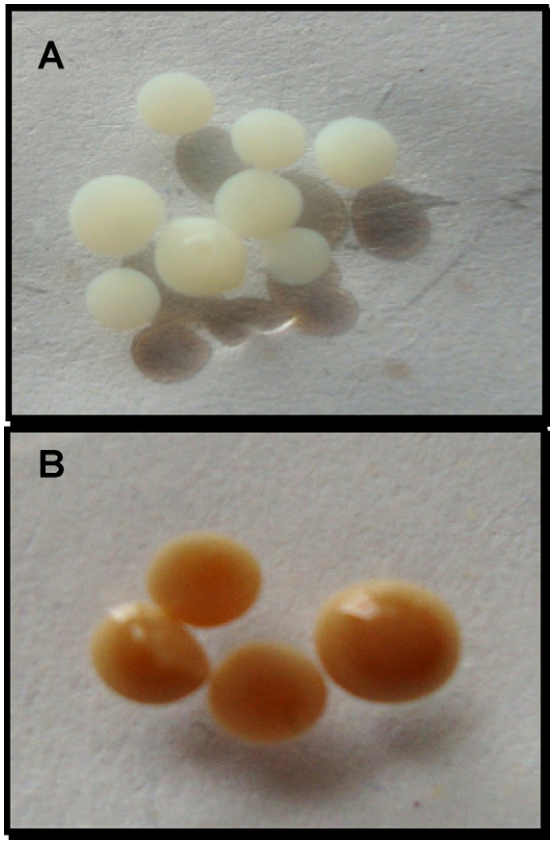

Fig. 1. (A) Picture of Chitosan beads (1.5%) in acetic acid solution (1.5%) by heating at 45 °C and (B) 2% glutaraldehyde activated Chitosan beads, using Sony W-220 cyber shot with 8 mega pixel at a resolution of 4×.

3. Results and discussion

3.1. Optimum conditions for α-amylase immobilization on Chitosan beads

Fig. 1 (A) and (B) shows Chitosan beads prepared with the method stated above and glutaraldehyde activated Chitosan beads, using Sony W-220 Cyber Shot with 8 mega pixel at resolution of 4×. The concentration of Chitosan was varied to ensure the final bead was mechanically tough to resist wear and tear or washing/storage conditions. We have used 1.5% Chitosan for our studies, though the bead formation was good above 1.5%, the diffusion of substrate inside the gel would be restricted. The use of glutaraldehyde also had an influence on the activation of the bead; 2% (v/v) glutaraldehyde resulted in maximum immobilization. With the increase of the concentration of the cross-linking reagent, the activation increased, and hence activity of the immobilized enzyme was increased. With the high enough increase of the concentration of cross-linking reagent, a large number of groups were activated. Space obstruction appeared due to multipoint attachment of enzyme with the activated carrier and hence, activity of the immobilized enzyme was decreased.

The protein concentration was also varied and the optimal immobilization was obtained with 6 mg and above, which did not result in significant increase showing that all the free aldehyde groups of glutaraldehyde were saturated with the enzyme or a great quantity of enzyme was immobilized and arranged closely on the Chitosan, which would make the enzyme overlap in space and affect

Table 1
(A) The conditions tested for optimal immobilization, (B) % immobilization obtained under different conditions for Chitosan beads and (C) % immobilization obtained under different conditions for Amberlite beads.

Conditions varied	Immobilization (%)	
(A) Conditions	(B) Chitosan beads	(C) Amberlite beads
Glutaraldehyde (%)		
1	32.4	20.4
2	**50.4**	37.8
2.5	46.8	**57.8**
3	40.8	45.0
Protein Amount (mg)		
3	24.6	64.8
4	43.0	62.4
5	52.4	**69.0**
6	**58.8**	54.4
Enzyme volume (μL)		
50	29.0	19.73
100	**52.4**	**58.4**
200	52.0	39.2
Incubation time (h)		
6	20.2	50.8
12	22.4	66.4
24	**62.0**	**70.4**
48	57.8	64.2

Bold values correspond to maximum % immobilization corresponding to the conditions specified.

the active center of the enzyme. As shown in Table 1, the best immobilization (62%) is obtained when 100 μL of protein (6 mg) was incubated for 24 h at room temperature. The beads showed linearity with respect to the activity, indicating homogenous distribution of the enzyme in the polymer.

3.2. Optimum conditions for α-amylase immobilization on Amberlite immobilization

As shown in the Table 1, various conditions were tested to obtain optimum immobilization. Amberlite MB-150 beads give maximum immobilization of 70.4% with 100 mg of Amberlite when activated with 2.5% (v/v) glutaraldehyde coupled with 100 μL of 5 mg soybean α-amylase for 24 h at 4 °C and pH 5.0. Since Amberlite is a mixed bed exchanger, pH plays an important role in binding of enzyme with the matrix along with glutaraldehyde. Below and above pH 5.0, a decrease in immobilization was observed (data not shown).

Fig. 2(A and B) shows microscopic image of control and glutaraldehyde activated soybean amylase immobilized onto Amberlite beads using Nikon light microscope (DS-Fi1) at a resolution of 10×. Immobilized beads clearly showed rough particles sticking on the whole surface of the Amberlite beads (Fig. 2B). The use of glutaraldehyde concentration had an influence on % immobilization (as shown in Table 1), 2.5% (v/v) glutaraldehyde had resulted in maximum immobilization. Glutaraldehyde when used at a concentration higher than 2.5% resulted in aggregation, precipitation, loss of enzyme activity and only fewer sites available for attachment to the matrix, thus posed practical problems and also inactivated amylase activity. There was no significant increase of percent immobilization observed when the amount of Amberlite was increased from 100 to 200 mg (data not shown). This was due to a limitation of surface area of the matrix available for enzyme immobilization.

3.3. Steady state kinetics

3.3.1. Optimum pH

The effect of pH on the activity of free and immobilized α-amylase on Chitosan and Amberlite is shown in Fig. 3. The pH

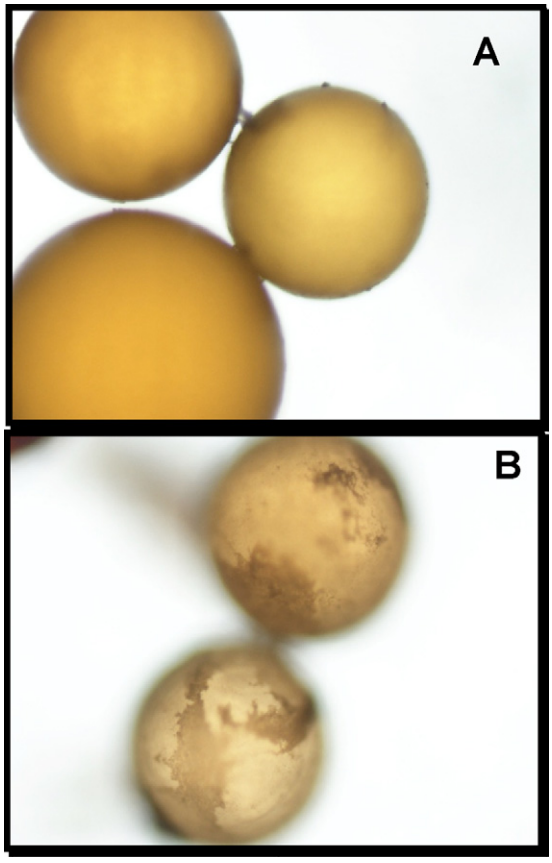

Fig. 2. (A) Microscopic image of glutaraldehyde activated (2.5%) soybean α-amylase coupled onto Amberlite MB-150 beads, using Nikon light microscope (DS-Fi1) at resolution of 10×. (B) Control Amberlite MB-150 beads.

optima of the soluble enzyme was 5.5 whereas, pH optima was 8.0 and 7.0 for enzyme immobilized on Chitosan and Amberlite MB-150, respectively. Immobilized α-amylase showed a shift towards the basic side and also broadening of the optimum pH resulting from the covalent binding of the enzyme with matrices. This could be due to the pH change in local environment of the bead and also due to diffusional constraints. The behavior of this enzyme

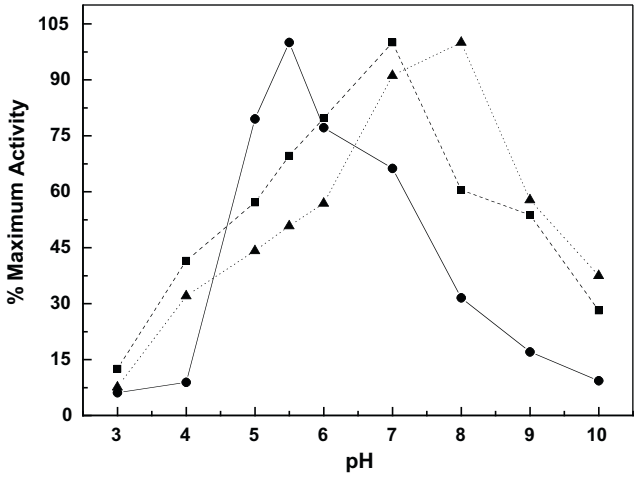

Fig. 3. Effect of pH on soluble (●) and immobilized [Chitosan (▲) and Amberlite (■)] α-amylase. The % maximum enzyme activity was determined by DNS method at various pH viz., 50 mM sodium acetate buffer (pH range 3.0–5.6), 50 mM phosphate buffer (pH range 5.7–8.0), 50 mM Tris buffer (pH range 8.0–10.0) with 1% soluble potato starch.

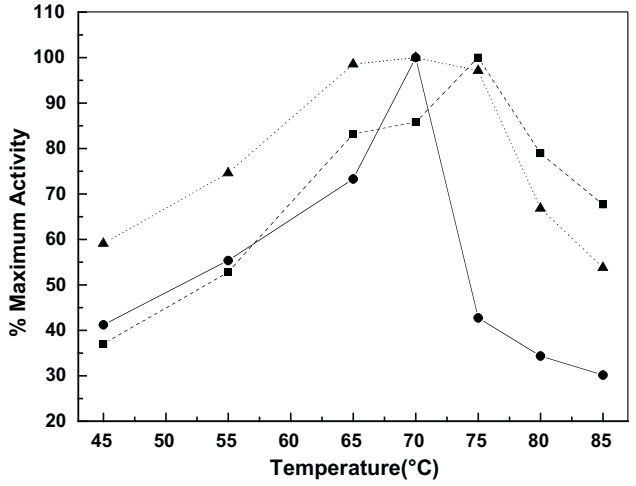

Fig. 4. Effect of temperature on soluble (●) and immobilized [Chitosan (▲) and Amberlite (■)] α-amylase. The % maximum enzyme activity was determined at various temperatures in standard buffer (for soluble) and 50 mM Tris buffer (for immobilized enzyme) at their optimal pH with 1% soluble potato starch as substrate.

molecule would have been modified by its immediate microenvironment. According to Mosbach [82], surface and residual charges on the solid matrix and the nature of the enzyme bound, the pH value in the immediate vicinity of the enzyme molecule may change and thus cause a shift in the pH optimum of the enzyme activity.

Recently α-amylase purified from mung bean showed a shift of 1.4 units towards the basic side upon immobilization on Chitosan and Amberlite [78]. Similarly, pigeonpea urease immobilized on Chitosan beads and jack bean urease immobilized on a fixed bed reactor showed a shift towards basic side [67]. No shift was observed when urease was immobilized onto Amberlite [72]. Pea β-galactosidase immobilized onto Amberlite MB-150 showed a broad pH optima in the range of 2.7–3.3 with ONPG but in the range of 3.9–4.4 with lactose, whereas, soluble β-galactosidase enzyme has sharp pH optima at pH 3.2 and 4.0 with ONPG and lactose as substrates, respectively [76]. A shift towards acidic region has been observed when α-amylase was immobilized on zirconium dynamic membrane and poly(methylacrylate-acrylic acid) microspheres [83,28]. The optimum pH of free and immobilized thermostable α-amylase on oxidized bagasse (a natural cellulosic material) at 40 °C was in the range of 6–7, and 7–9, respectively, and both free and immobilized enzyme was restricted to pH 7.0 in case of 90 °C [84].

3.3.2. Optimum temperature

Fig. 4 shows the effect of temperature on α-amylase immobilized on Chitosan and Amberlite MB-150. Soluble α-amylase from soybean has an optimum temperature of 70 °C whereas; immobilized α-amylase showed an optimum temperature of 70 °C and 75 °C for Chitosan and Amberlite, respectively. There is no change in optimum temperature for α-amylase immobilized onto Chitosan, thus indicating that there were no structural changes induced upon immobilization and the matrix also did not afford protection from heat. The small increase in the optimum temperature for Amberlite amylase may arise from changing the conformational integrity of the enzyme structure by covalent bond formation via amino groups. Similar results were obtained for Chitosan and Amberlite immobilized mung α-amylase, which showed maximum activity at 75 °C and 65 °C, respectively [78]. α-Amylase from *Bacillus circulans* immobilized on calcium alginate beads showed an increase in operating temperature [85]. For α-amylase, shifts towards both higher and lower temperatures were reported [44,83]. Thermostable α-amylase immobilized on oxidized bagasse showed optimum temperature of 90 °C and 95 °C, for free and immobilized enzymes, respectively [84].

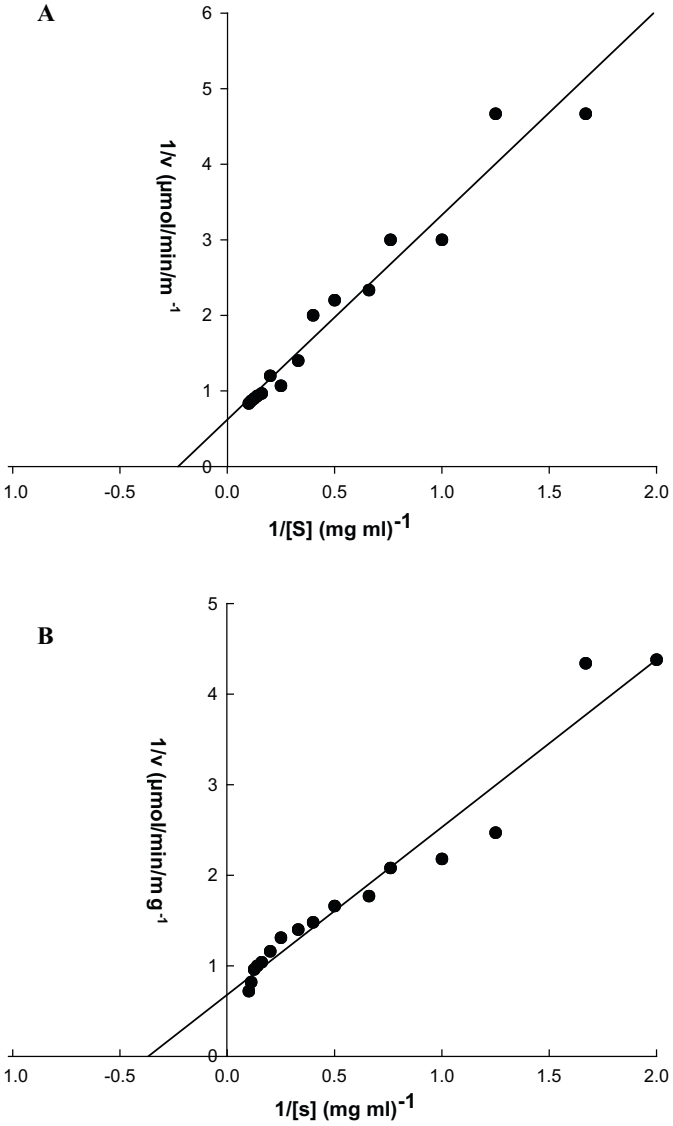

Fig. 5. (A) Determination of K_m for immobilized Chitosan and (B) Amberlite α-amylase by Lineweaver–Burk plot method at 37 °C. Starch concentration varied from 0 to 10 mg/mL in 50 mM Tris buffer at their optimal pH.

3.3.3. K_m and V_{max}

K_m and V_{max} for Chitosan and Amberlite immobilized α-amylase were calculated using Lineweaver–Burk plot with starch as substrate; as shown in Fig. 5A and B. The K_m was found to be 4 mg/mL and 2.5 mg/mL for α-amylase immobilized on Chitosan and Amberlite, respectively. We earlier reported K_m for the soluble α-amylase to be 0.71 mg/mL [77]. V_{max} was found to be 1.25 μmoL (reducing sugar i.e. maltose)/min/mg for α-amylase immobilized in Chitosan as well as Amberlite; it was found to be 2 μmol (reducing sugar i.e. maltose)/min/mg for soluble amylase (data not shown). The insignificant change of K_m could be due to fact that the conformational changes in tertiary structure of amylase and steric effects resulting from limitation of the accessibility of substrate to the active site are affected on immobilization and hence there is reduction in catalytic efficiency and an increase of K_m [86]. Chen et al. [29] also reported an increase in K_m, which clearly indicates an apparent low affinity of the enzyme towards its substrate compared to the soluble enzyme. Recently, Tripathi et al. [78] reported an apparent

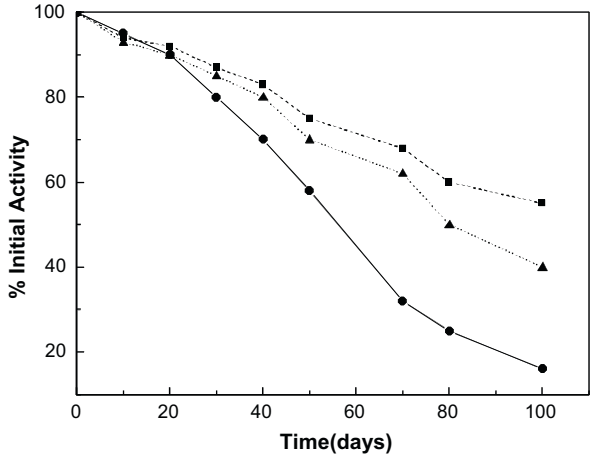

Fig. 6. Storage stability of soluble (●) and covalently immobilized [Chitosan (▲) and Amberlite (■)] α-amylase at 4 °C in standard buffer (for soluble) and 50 mM Tris buffer (for immobilized enzyme) at their optimal pH (also see Section 2).

K_m of 2.77 mg/mL for Amberlite and of 5 mg/mL for Chitosan, which is approximately 4 times higher than that of soluble α-amylase. A similar change was also observed in case of pigeonpea urease immobilized onto Chitosan beads [67].

3.4. Storage stability and reusability

The stability of α-amylase enhanced quite significantly upon immobilization. Higher stability of Amberlite beads was due to higher local enzyme concentrations. Orientation and crowdedness of Chitosan beads also effect % immobilization as lesser surface area is available for enzyme immobilization compared to Amberlite MB-150 beads. Improved storage stability by immobilization has been reported by various workers [87,88]. Chitosan beads stored at 4 °C showed practically no leaching of enzyme over a period of two weeks. The loss of activity, for free amylase after 100 days of storage at 4 °C was 85% and in comparison to Chitosan and Amberlite α-amylase the losses were 60% and 45%, respectively during the same period (Fig. 6).

With repeated use, the strength of binding between the matrix and enzyme is weakened, leading to leaching of enzyme from the matrix and loss in activity. Moreover, frequent encountering of substrate in the active site causes its distortion, thus reducing catalytic efficiency. The Chitosan amylase showed a residual activity of 38% and Amberlite-amylase showed residual activity of 58%, after 10 reuses (Fig. 7).

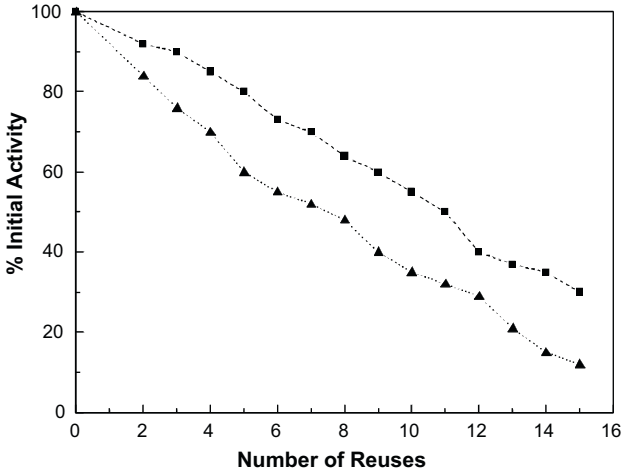

Fig. 7. Reusability (10 uses) of immobilized Chitosan and (▲) and Amberlite (■) α-amylase at 4 °C in 50 mM Tris buffer at their optimal pH (also see Section 2).

4. Conclusion

α-Amylase was successfully immobilized onto Chitosan and Amberlite using covalent binding. The immobilized Chitosan beads showed good linearity with respect to activity thereby indicating the homogenous distribution of the enzyme inside the polymer. Reaction is mild and easy and can be scaled up for industrial applications. The immobilized enzyme was stable on storage compared to the soluble enzyme under similar conditions and immobilized α-amylase showed no leaching of enzyme over a period of one month. Bacterial amylases, amyloglucosidase, α-amylases are probably the most commonly used enzymes in the starch industry and are relatively inexpensive. By replacing an easy soluble amyloglucosidase with environment friendly immobilized enzyme, it is possible to reduce the conversion time of saccharification and can be used many times. Immobilization of α-amylase on Chitosan and Amberlite makes the enzyme more useful in starch hydrolysis and for various industrial applications. Immobilized α-amylases can be used as drug-design targets for the potential development of compounds for the treatment of diabetes, obesity and hyperlipaemia.

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