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Cost effective surface functionalization of silver nanoparticles for high yield immobilization of *Aspergillus oryzae* β-galactosidase and its application in lactose hydrolysis

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ABSTRACT

The present study demonstrates synthesis, characterization and surface functionalization of silver nanoparticles (AgNPs) via glutaraldehyde for high yield immobilization of *Aspergillus oryzae* β-galactosidase. Soluble β-galactosidase (SβG), enzyme adsorbed on unmodified AgNPs (UβG) and surface modified AgNPs (MβG) showed same pH-optima at pH 4.5. However, it was observed that MβG exhibited enhanced pH stability toward acidic and alkaline sides, and increased temperature resistance as compared to SβG and UβG. Michaelis constant, K_m was increased nearly three-folds for MβG while V_{max} for soluble and MβG was 0.515 mM/min and 0.495 mM/min, respectively. Furthermore, MβG showed greater resistance to product inhibition mediated by galactose as compared to it soluble counterpart and exhibited excellent catalytic activity even after its fourth successive reuse. The remarkable bioconversion rates of lactose from milk in batch reactors further revealed an attractive catalytic efficiency of β-galactosidase adsorbed on surface functionalized AgNPs thereby promoting its use in the production of lactose free dairy products.

1. Introduction

Nanotechnology is gaining tremendous attention in the present century due to its capability of modulating metals into their nanosize which changed their chemical and physical properties drastically. Moreover, the fabrication technique has been exploited in the recent past to modify these nanostructured materials for extending their applications in diverse fields [1–3]. The surface functionalized nanoscaled fibers offered various advantages like high surface area to volume ratio and tunable porosity which enabled nanotechnologists to manipulate their composition with desired function [4,5]. Firstly, surface modification stabilizes the nanoparticles against agglomeration. Secondly, this technology rendered nanostructured materials compatible with another phase, avoids homogeneity and compatibility problems between two phases thereby improving their mechanical properties. A third interest in nanoparticle modification is to enable their self-organization [6,7].

The antibacterial activity of AgNPs is known since ancient times and its non-toxicity to human cells in lower concentrations has also been demonstrated in the recent past. Moreover, the electronic industry is also exploring the use of colloidal silver inks for printing circuit components in flexible electronic applications for which compliant electrodes are needed. Since AgNPs are bioactive and have broad spectrum antimicrobial properties, they are widely incorporated into wound dressings and are used as an antiseptic/disinfectant in medical applications and in consumer goods [8–10]. Nevertheless, AgNPs are highly selective and efficient catalysts that promises great advancements in various applications such as chemical conversions, biosensing and bioremediation [11,12]. However, their widespread utilization is often limited by low operational stability. Therefore, there have been many studies to stabilize and reduce their toxicity via surface modification in order to exploit them for enzyme immobilization, enzyme modification, genetic modification and medium engineering [13,14]. Therefore, immobilization of biocatalysts on surface modified nanocomposites helps in their economic re-use and in the development of continuous bioprocesses which offered the

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possibility of wider and more economical exploitation of biocatalysts in industry, waste treatment and medicine [15–17]. Moreover, the functionalized matrix may serve as a shield for harsh environmental conditions like pH variation, temperature alteration and shaking condition [18,19].

Recently, surface functionalization of AgNP has attracted major attention of biologists due to their plasmonic properties and easy surface chemistry. Thus, chemical alteration of their surface properties is being actively explored with polymers, biological ligands and macromolecules [20–22]. Phospholipid derivatives containing disulfide groups were used to modify AgNP surfaces in order to enhance their biocompatibility and cell affinity for biosensing and drug delivery applications [14]. Earlier, Lee and co-workers have reported a novel strategy for preparing AgNP–oligonucleotide conjugates which were based upon DNA with cyclic disulfide-anchoring groups. This finding provided vital approaches for exploiting AgNP–oligonucleotide conjugates in several applications like molecular diagnostic labels, synthons in programmable material synthesis and functional components for nanoelectronic and plasmonic devices [23]. In another study, glucose oxidase was covalently immobilized on surface of thiol-modified AgNPs and it exhibited greater stability at higher temperature and pH range as compared to the soluble enzyme [12].

β-Galactosidase (EC 3.2.1.23) promises great potential interest in dairy industry and is produced by animals, plants and microorganisms [24,25]. It catalyzes the *in vivo* hydrolysis of lactose into corresponding monosaccharides, glucose and galactose. Moreover, several new applications such as hydrolysis of different saccharides with galactose present in their structures or biosynthesis of oligosaccharides have been proposed [26].

In this study, a simple procedure has been investigated to immobilize *Aspergillus oryzae* β-galactosidase on glutaraldehyde modified silver nanoparticles for studying its potential biotechnological application in lactose hydrolysis.

2. Materials and methods

2.1. Materials

A. oryzae β-galactosidase (activity = 1200 U/mg), silver nitrate, glutaraldehyde, 2-nitrophenyl β-D-galactopyranoside (ONPG), bovine serum albumin, lactose and glucose oxidase–peroxidase assay kit were obtained from Sigma Chem. Co. (St. Louis, MO, USA). Sodium borohydride and aniline was purchased from SRL Chemicals (Mumbai, India). All reagents were prepared in double distilled water with chemicals of analytical grade.

2.2. Synthesis and characterization of AgNPs

AgNPs were synthesized according to the procedure described by Solomon et al., 2007 [27]. $AgNO_3$ (1.0 mM) was prepared in distilled water and the solution was magnetically stirred in ice bath for 15 min. Sodium borohydride (2.0 mM) was then mixed in stirring condition which resulted in the change of color from transparent to golden yellow thereby indicating the formation of AgNPs. X-ray diffraction analysis of the synthesized AgNPs was investigated by Rigaku Miniflex X-ray diffractometer with Cu-Kα radiation (λ = 1.54060 Å) in 2θ ranging from 30° to 80° to observe its structure and lattice parameters. The average size of crystal (D) was calculated by Scherer's formula from 111th line width of XRD peak. UV–vis spectra of AgNPs were performed in n-methyl 2-pyrolodine and the absorbance was taken as a function of wavelength ranging from 270 to 800 nm by Perkin Elmer Spectrophotometer. Moreover, the obtained AgNPs were characterized using Dynamic Light Scattering in a Malvern Zetasizer Nano ZS while the morphology and size of AgNPs was observed with a JEOL JEM-2100F transmission electron microscope with an accelerating voltage of 15 kV at All India Institute of Medical Sciences, India. Sample for TEM analysis was prepared by drop coating diluted NP solution on carbon-coated copper grids at normal atmospheric conditions.

2.3. Surface functionalization of AgNPs

Activation of AgNPs was achieved by glutaraldehyde with slight modification according to the procedure described by Verma et al. [5]. Initially, the nanoparticles were washed twice with deionized water. AgNPs were then recovered by centrifugation (2000 rpm for 10 min). The washed nanoparticles were then suspended in 0.5 M glutaraldehyde. Support activation was carried in a shaker (150 rpm for 6 h). The activated support was removed by centrifugation, washed thrice with 30 mL of deionized water to remove traces of glutaraldehyde and subsequently washed with enzyme assay buffer (0.1 M sodium acetate buffer, pH 4.5) and used for further studies.

2.4. Toxicity of glutaraldehyde modified AgNPs by plasmid nicking assay

Plasmid nicking assay was performed with slight modifications by pUC18 DNA according to the procedure described by Ansari et al. [16]. Reaction mixture (30 μL) containing 10 mM Tris–HCl buffer (pH 7.5), pUC18 DNA plasmid (0.8 μg) and surface functionalized AgNPs (15 μL) were incubated for 2 h at 37 °C. Fifteen microlitre of a solution containing 40 mM EDTA, 0.05% bromophenol blue (tracking dye) and 50% (v/v) glycerol was added after incubation and the solution was then subjected to electrophoresis in submarine 1.0% agarose gel. Ethidium bromide stained gel was then viewed and photographed on a UV-transilluminator.

2.5. Immobilization of β-galactosidase on surface functionalized AgNPs

β-Galactosidase (2400 U) was mixed with unmodified and modified AgNPs (10 mg each) independently and the resulting mixture was stirred overnight on a magnetic stirrer at 30 °C in sodium acetate buffer (0.1 M, pH 4.5). The precipitate was collected after centrifugation at 2000 rpm for 20 min. Enzyme adsorbed on unmodified AgNPs (UβG) and surface modified AgNPs (MβG) was washed thrice with assay buffer and finally suspended and stored at 4 °C in the assay buffer for further use.

2.6. Enzyme assay of soluble and immobilized β-galactosidase

The activity assay of soluble and immobilized β-galactosidase was performed according to the procedure described by Ansari and Husain [24] with slight modification. The reaction was carried with continuous agitation in an assay volume of 2.0 mL containing 1.7 mL of 0.1 M sodium acetate buffer (pH 4.5), 0.1 mL suitably diluted 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 the product formed was measured spectrophotometrically at 405 nm. All experiments were performed in triplicates and the mean values were expressed as ±SD.

It should be noted that one unit (1.0 U) of β-galactosidase activity is defined as the amount of enzyme that liberates 1.0 μmole of *o*-nitrophenol (ε_m = 4500 L/mol/cm) per min under standard assay conditions.

2.7. Biochemical characterization

Enzyme activity of free and immobilized β-galactosidase (2.0 U) was assayed in buffers of different pH (pH 3.0–9.0, 0.1 M) at 50 °C. The buffers used were glycine–HCl (pH 3.0), sodium acetate (pH 4.0 and 5.0), sodium phosphate (6.0 and 7.0) and Tris–HCl (pH 8.0 and 9.0). The activity at pH 4.5 was taken as control (100%) for the calculation of remaining percent activity at other pH.

Effect of temperature on soluble and immobilized enzyme was determined by measuring the relative enzyme activity in the temperature range of 20–80 °C at pH 4.5. Other experimental conditions adhered to the standard assay protocol. The activity at 50 °C was taken as control for calculating the relative activity of free and immobilized enzyme at other temperatures.

2.8. Thermal stability

Thermal stability of free and immobilized β-galactosidase at selected temperature was quantified in terms of loss in enzyme activity when incubated at respective temperatures in the absence of substrate. Thus, soluble and immobilized β-galactosidase was incubated at 60 °C in 0.1 M sodium acetate buffer (pH 4.5) for varying times. Aliquots of each preparation (20 μL) were taken at indicated time intervals and chilled quickly in crushed ice for 5 min. The enzyme was brought at room temperature. After keeping the samples for certain period of time, residual β-galactosidase activity was determined in the manner described above. The relative activity of free and immobilized β-galactosidase without incubation was defined as control and arbitrarily attributed 100% relative activity for each of their respective reactions.

2.9. Determination of kinetic parameters

Kinetic parameters of SβG, UβG and MβG was determined (from Line–Weaver Burk plot) by measuring their initial rates at varying concentrations of ONPG in working buffer at pH 4.5.

2.10. Reusability

Reusability of UβG and MβG was assessed at 50 °C in 0.1 M sodium acetate buffer (pH 4.5) by carrying out the hydrolysis of ONPG under standard assay conditions. After each cycle, immobilized enzyme was removed by centrifugation at 2000 rpm for 5 min. Immobilized β-galactosidase was collected and washed simultaneously with deionized water and assay buffer. In running the second cycle, immobilized

enzyme was redissolved in fresh buffer and added to fresh ONPG. Activity of immobilized enzyme after first cycle was considered as control and attributed a relative activity of 100%. Each cycle is defined here as the complete hydrolysis of substrate present in a reaction mixture.

2.11. Storage stability

Soluble and immobilized β galactosidase was stored at 4 °C in 0.1 M sodium acetate buffer, pH 4.5 for 2 months. Aliquots from each preparation (20 μL) were taken in triplicates at the gap of 10 days and were then analyzed for the activity. The activity determined on the first day was taken as control (100%) for the calculation of remaining percent activity.

2.12. Lactose hydrolysis

Lactose from milk and whey (500 mL) was independently incubated with free and immobilized β-galactosidase, and stirred continuously for 10 h at 50 °C in a shaking water bath. Aliquots were taken at regular interval and lactose hydrolysis was measured according to the procedure described by Verma et al. [5].

2.13. Estimation of protein

Protein concentration was determined by dye binding method [28].

2.14. Statistical analysis

Each value represents the mean for three independent experiments performed in triplicates, with average standard deviation <5%. The data expressed in various studies were plotted using Sigma Plot-9 and expressed with standard deviation of error (±). A Student's *t*-test was used to examine statistically significant differences. Analysis of variance was performed using ANOVA, *P* values <0.05 were considered statistically significant.

3. Results and discussion

AgNPs are important for chemistry, physics and biology due to their unique optical, electrical and photothermal properties. They are widely used to impart stability to various bioactive substances including peptides, enzymes, antibodies and DNA due to their greater porosity and interconnectivity for enzyme immobilization as compared to other nanostructured supports [13,15,19]. This manuscript addresses the recent advancement in exploiting AgNPs as a host for immobilization of *A. oryzae* β-galactosidase and made a detailed comparison between soluble enzyme and enzyme immobilized on unmodified and glutaraldehyde modified AgNPs. The prospective applications of surface functionalized AgNPs in the development of lactose-free dairy products are also discussed.

Fig. 1 showed a typical X-ray diffraction (XRD) pattern of AgNPs at room temperature. XRD diffraction pattern showed its cubic crystal symmetry and face centered lattice structure. All the peaks were similar to cubic structure. The lattice parameters calculated from XRD pattern was $a = 4.086$. The observed 2θ values are in accordance with standard JCPDS No. 04-0783. Scherer's formula was used to determine the particle size (D) of the sample: $D = 0.9\lambda/B\cos\theta$, where λ is the X-ray wavelength (1.54060 Å), B is the full width at half-maximum of silver (1 1 1) line and θ is the diffraction angle. The particle size of sample was found to be ~26 nm which was estimated from 1 1 1 line width of the XRD peak. UV–vis spectrum of AgNPs showed an absorption peak near 400 nm which is a characteristic peak for AgNPs (Fig. 2). These nanoparticles showed λ_{max} value at 394 nm which is in good agreement with previous results [29]. Dynamic light scattering (also known as Photon Correlation Spectroscopy or Quasi-Elastic Light Scattering) was used to determine the size distribution profile of AgNPs. The average mean size of AgNPs was observed as 26.6 nm (Fig. 3). TEM image of AgNPs is shown in Fig. 4 which depicts that they were very fine particles having a mean diameter of 26 nm. Moreover, the damaging effect of surface functionalized AgNPs on plasmid DNA was found to be negligible since the band intensity of treated sample was similar to that of control (Fig. 5).

Glutaraldehyde is an important crosslinking agent that is widely used to crosslink enzymes for their enhanced storage stabilization and better performance at high temperature and pH [5,30,31]. It has also been used in the recent past to provide genuine enhancement of conformational stability to α-galactosidase from *Thermus* sp. In this case, supports activated with 1% (v/v) glutaraldehyde

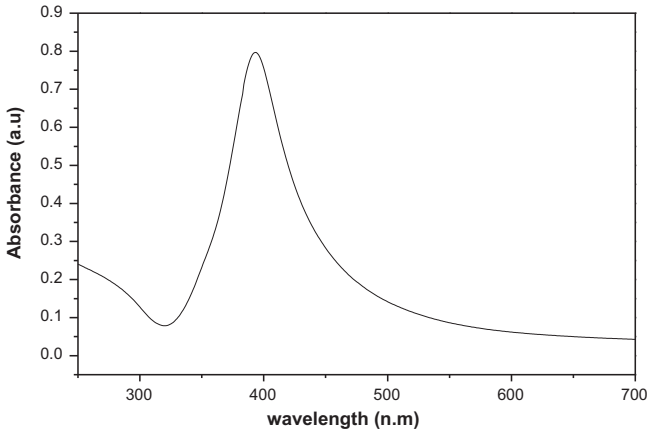

Fig. 2. UV spectra of AgNPs. UV spectra of AgNPs was performed in n-methyl 2-pyrolodine and the absorbance was taken as a function of wavelength ranging from 270 to 800 nm by Perkin Elmer Spectrophotometer.

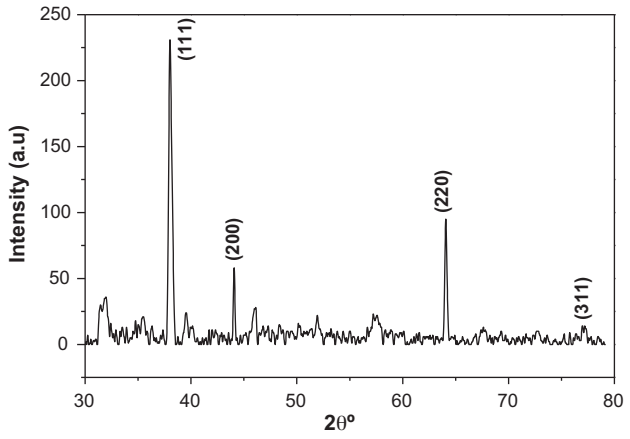

Fig. 1. XRD pattern of AgNPs. X-ray diffraction pattern of AgNPs was recorded at room temperature using Rigaku Miniflex X-ray diffractometer with Cu-Kα radiation (λ = 1.54060 Å) in 2θ ranging from 30° to 80°.

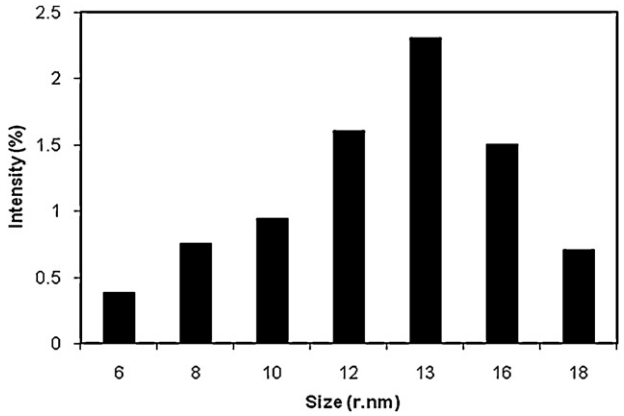

Fig. 3. Differential Light Scattering of surface functionalized AgNPs. Glutaraldehyde functionalized AgNPs were characterized by Dynamic Light Scattering in a Malvern Zetasizer Nano ZS to observe their mean average size.

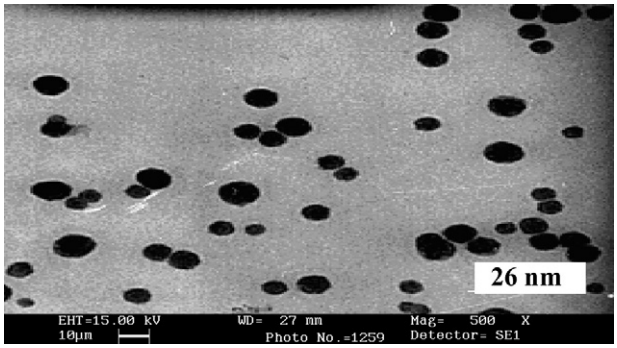

Fig. 4. Transmission electron microscopy of AgNPs. Morphological analysis of AgNPs was performed by JEOL JEM-2100F transmission electron microscope. Sample for TEM analysis was prepared by drop coating diluted NP solution on carbon-coated copper grids at normal atmospheric conditions.

Fig. 5. Plasmid nicking toxicity assay for glutaraldehyde modified AgNPs. Toxicity of surface functionalized AgNPs was analyzed by plasmid nicking assay as given in the text. Circular double stranded pUC18 DNA was used. Lane (C) denotes control pUC18 DNA while lane (T) signifies pUC18 DNA incubated with modified AgNPs. Ethidium bromide stained gel was used on a UV-transilluminator for this purpose.

led to retention of 90% initial activity after 48 h at pH 7.0 and 75 °C while the soluble enzyme was fully inactivated within 8 h under similar incubation conditions [32]. Thus, in the present manuscript, AgNPs were surface modified with glutaraldehyde to

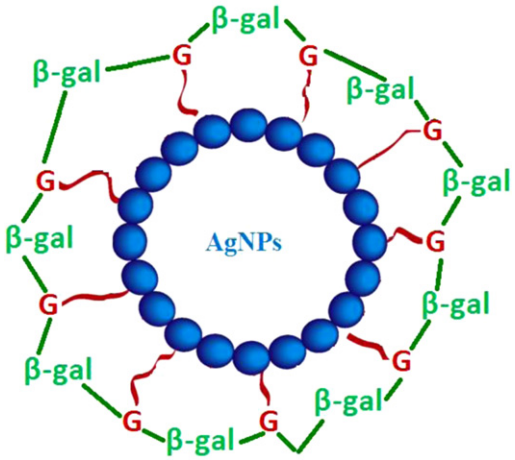

Fig. 6. Schematic diagram for immobilization of β-galactosidase on surface functionalized AgNPs. A probable mechanism for surface functionalization of AgNPs with glutaraldehyde and immobilization of β-galactosidase on the surface modified nanomatrix.

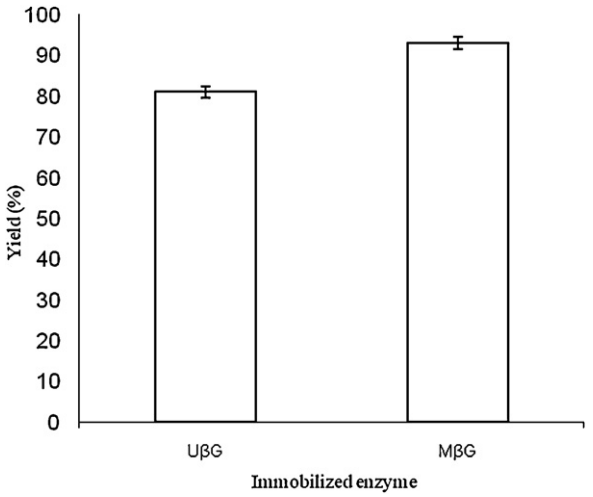

Fig. 7. Immobilization yield. β-galactosidase was mixed with unmodified and modified AgNPs as described in the text. The precipitate was collected after centrifugation at 2000 rpm for 20 min and enzyme adsorbed on unmodified AgNPs (UβG) and surface modified AgNPs (MβG) was washed thrice with assay buffer for calculating the immobilization yield.

immobilize β-galactosidase (Fig. 6). Fig. 7 demonstrates that glutaraldehyde functionalized AgNPs retained significantly very high enzyme activity (93%) as compared to 81% activity yield obtained in case of unmodified AgNPs.

MβG showed greater activity at high and low pH values as compared to its soluble counterpart (Fig. 8). It could be due to the fact that greater alteration/distortion was produced in the tertiary structure of free enzyme in highly acidic and basic solutions as compared to the immobilized enzyme [26]. However, the loss in enzyme activity was noticed at higher temperatures due to denaturation of enzyme molecules which resulted in the rupturing of polypeptide chain and degradation of polymer matrix. Thus, the immobilized enzyme was significantly stabilized at higher temperatures (Figs. 9 and 10).

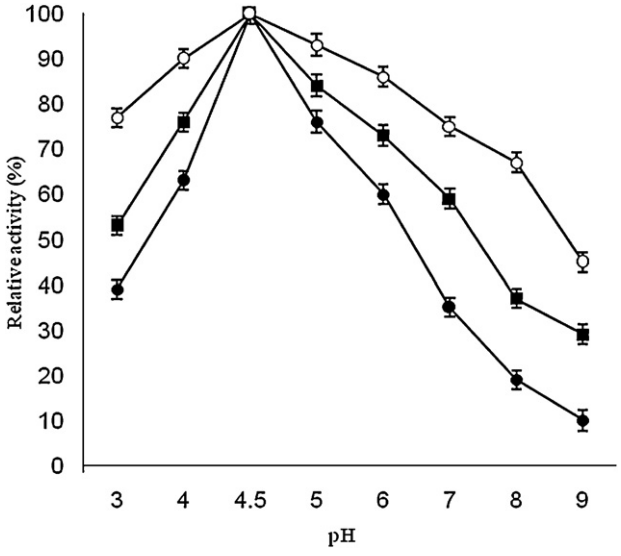

Fig. 8. pH activity profiles for soluble and immobilized β-galactosidase. The activity of soluble and immobilized β-galactosidase (2.0 U) was measured in the buffers of various pH (3.0–9.0, 0.1 M). The buffers used were glycine–HCl (pH 3.0), sodium acetate (pH 4.0, 5.0), sodium phosphate (6.0, 7.0) and Tris–HCl (pH 8.0, 9.0). The activity at pH 4.5 was taken as control (100%) for the calculation of remaining percent activity. The symbols show activity of SβG (●), UβG (■) and MβG (○).

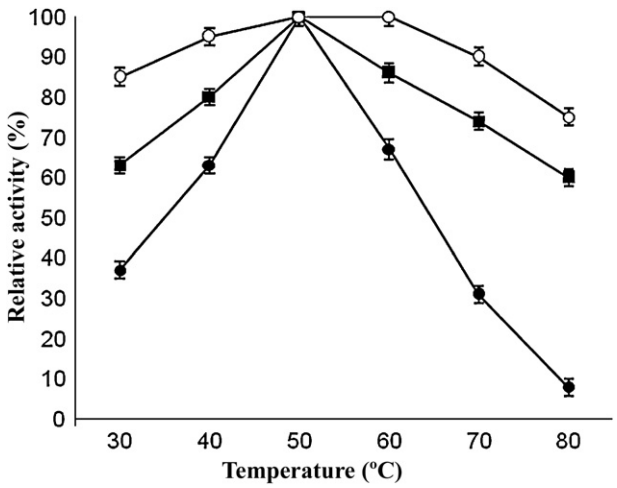

Fig. 9. Temperature activity profiles for soluble and immobilized β-galactosidase. The activity of soluble and immobilized β-galactosidase (2.0 U) was assayed in 0.1 M sodium acetate buffer (pH 4.5) at various temperatures (30–80 °C) for 15 min. The activity obtained at 50 °C was considered as control (100%) for the calculation of remaining percent activity. For symbols, refer to figure legends 8.

In order to construct enzymatic devices, major emphasis is given on the control of enzyme activity in presence of inhibitors and on the reaction kinetics, which is highly dependent on the interface between the nanocomposites and enzyme. Thus, the stability of MβG was investigated in the presence of galactose as an inhibitor (Fig. 11). It showed pronounced activity at higher concentration of galactose as compared to UβG and SβG. Moreover, two and a half fold increase in K_m value was observed for MβG as compared to SβG (Table 1). The changes in kinetic parameters suggested that covalent binding of enzyme onto the glutaraldehyde-activated AgNPs resulted in change of affinity for the substrate. It was due to the fact that some conformational changes occurred in the active site of immobilized enzyme and thus it required more substrate to saturate the active site of enzyme. Moreover, some of the active centers get blocked after immobilization which leads to reduction in

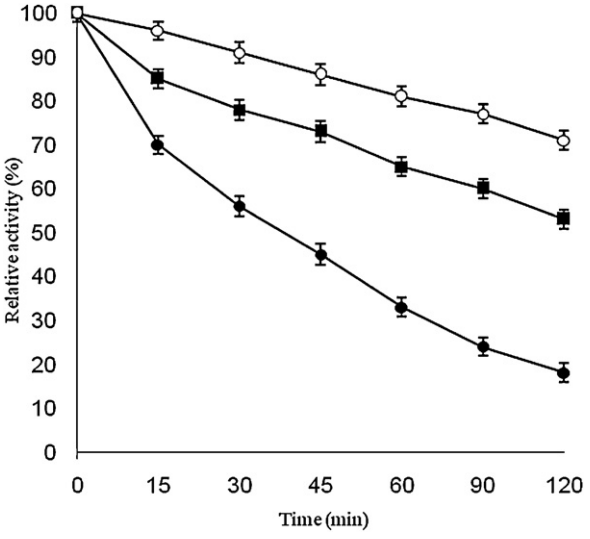

Fig. 10. Thermal denaturation of soluble and immobilized β-galactosidase. Soluble and immobilized β-galactosidase (2.0 U) was incubated at 60 °C in 0.1 M sodium acetate buffer (pH 4.5) for varying times. Aliquots of each preparation (20 μL) were taken out at indicated time intervals and chilled quickly in crushed ice for 5 min. The activity of the enzyme obtained without incubation at 60 °C was taken as control (100%) for the calculation of remaining percent activity. For symbols, refer to figure legends 8.

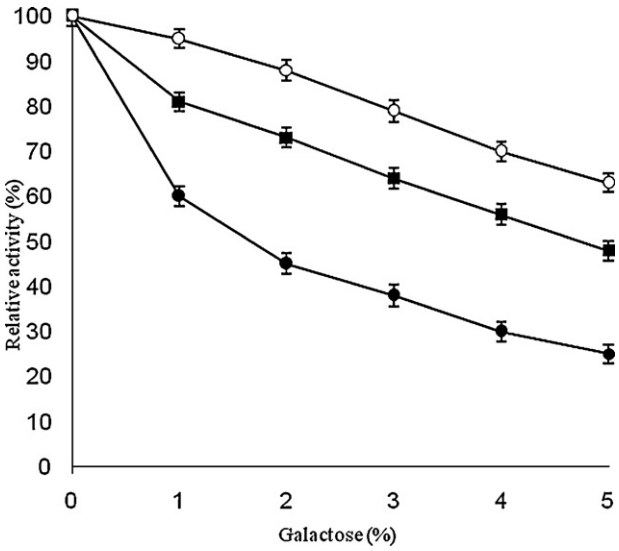

Fig. 11. Effect of galactose on soluble and immobilized β-galactosidase. Effect of galactose on soluble and immobilized β-galactosidase (2.0 U) was measured in the presence of increasing concentrations of galactose (1.0–5.0%, w/v) in 0.1 M sodium acetate buffer (pH 4.5) for 1 h at 37 °C. The activity of enzyme without added galactose was considered as control (100%) for the calculation of remaining percent activity at various concentrations of galactose. For symbols, refer to figure legends 8.

reaction rate considerably as observed by decrease in its V_{max} value [16,26]. Since the covalent coupling of enzyme onto the modified nanocomposites leads to its favored orientation on the matrix, this technique will prove suitable for anchoring the enzyme close to the electrode surface in constructing lactose based biosensors with greater preservation of biological activity.

Greater percent of lactose hydrolysis was observed for SβG during initial hours as compared to MβG (Fig. 12) because soluble enzyme was more accessible for the hydrolysis of lactose during initial few hours but after prolong incubation, rate of lactolysis by SβG decreased much faster due to product inhibition of enzyme [33]. It should be noted that the rate of hydrolysis of lactose in whey and milk depends on the activity of β-galactosidase, which in turn depends on reaction conditions like pH, heat, concentration of enzyme and processing time [34]. Here, results indicated that higher lactose hydrolysis was obtained in whey than in skimmed milk, it can be attributed to the fact that pH of whey lies in the range of 4.5–5.0 whereas milk has a pH of 6.5–6.8. *A. oryzae* β-galactosidase showed 100% activity at pH 4.5 since it has an acidic pH-optimum and its activity decreased considerably at higher pH [24]. β-galactosidase from *A. oryzae* has been therefore preferred to hydrolyze lactose in whey due to its acidic pH-optima and high thermal stability. It has been reported earlier that 50% lactose hydrolysis was achieved in 4 h by *Kluyveromyces lactis* (2 U/mL) and *Kluyveromyces marxianus* (150 U/100 mL) β-galactosidase by Verma et al. [5] and Puri et al. [35], respectively. Similarly, recombinant *Pseudoalteromonas* sp. 22b β-galactosidase immobilized on chitosan beads (7.5 U/g of lactose) showed 58% lactose hydrolysis in 10 h at 15 °C [36] and *A. oryzae* β-galactosidase immobilized

Table 1
Kinetic parameters for soluble and immobilized β-galactosidase.

Enzyme preparation	Kinetic constants	
	K_m (mM)	V_{max} ($\times 10^{-4}$ mol/min)
SβG	2.30 ± 1.48	0.515 ± 0.79
UβG	5.18 ± 0.31	0.504 ± 1.32
MβG	6.08 ± 0.69	0.495 ± 0.93

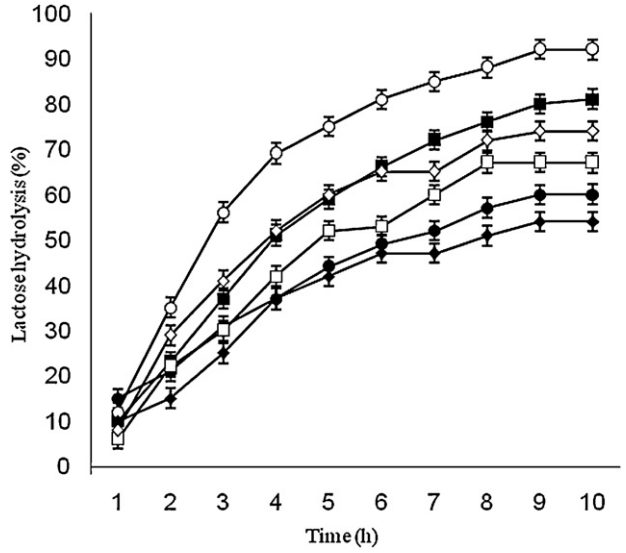

Fig. 12. Hydrolysis of milk and whey lactose by soluble and immobilized β-galactosidase in batch process. Hydrolysis of lactose from whey is shown by the symbols SβG (●), UβG (■) and MβG (○) while the hydrolysis of milk lactose is shown by symbols SβG (♦), UβG (□) and MβG (◊).

on zinc oxide nanoparticles (400 U/500 mL) exhibited maximum milk lactolysis, 71% after 8 h [37]. MβG showed pronounced lactose hydrolysis of 92% and 74% from whey and milk, respectively even after 10 h, thus this preparation could find promising application in converting lactose from milk and whey in batch reactors at higher temperatures.

Reusability experiment illustrated that MβG retained 88% activity even after its 6th repeated use (Fig. 13) while 93% of its original activity was observed even after 2 months storage at 4 °C (Table 2). In view of its cost effectiveness, excellent stability against physical and chemical denaturants, and improved reusability and stability, glutaraldehyde modified AgNPs bound β-galactosidase may find its applications for continuous conversion of lactose from other dairy products for longer durations in a reactor.

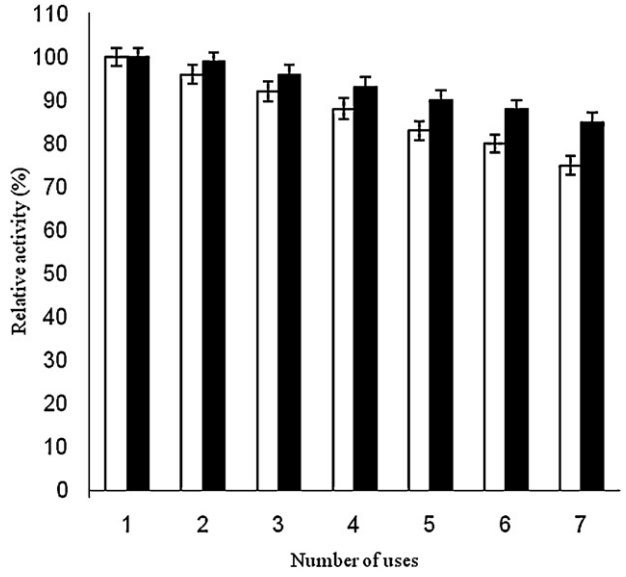

Fig. 13. Reusability of immobilized β-galactosidase. Reusability of immobilized β-galactosidase was monitored for 7 successive days. The preparation was taken in triplicates and was assayed for the remaining percent activity. The activity determined on the first day was taken as control (100%) for the calculation of remaining activity after each use. For symbols, refer to figure legends 8.

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

Number of days	Remaining activity (%)		
	SβG	UβG	MβG
Control	100	100	100
10	91 ± 2.66	98 ± 1.39	100
20	75 ± 2.51	93 ± 2.04	100
30	63 ± 1.93	87 ± 1.52	98 ± 1.34
40	55 ± 2.59	80 ± 1.36	97 ± 1.85
50	48 ± 2.18	76 ± 1.92	95 ± 1.20
60	43 ± 0.95	72 ± 2.42	93 ± 1.52

4. Conclusion

This work provided a highly efficient and different approach to immobilize *A. oryzae* β-galactosidase on glutaraldehyde modified AgNPs as nanomatrix. Immobilized enzyme showed enhanced stability against various physical and chemical denaturants thereby extending its applicability as a reusable and robust biocatalyst. Such type of novel support could serve as a potential enzyme immobilization carrier for other industrial applications.

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