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Enhanced Stability of α-Amylase via Immobilization onto Chitosan-TiO_2 Nanocomposite

V.U. Bindu[1] and P.V. Mohanan[2*]

[1,2]*Department of Applied Chemistry, Cochin University of Science and Technology, Kochi, Kerala, India*

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***Corresponding author:** P.V. Mohanan, Assisant Professor, Department of Applied Chemistry, Cochin University of Science and Technology, Kochi 22, Kerala, India, Tel.: 9447184874; E-mail: mohan@cusat.ac.in*

Abstract

The purpose of the present study is to enhance the stability properties of α-amylase by immobilization onto chitosan-TiO_2 nanocomposite. The immobilization of enzyme onto the nanocomposite was done by adsorption method. α-amylase is an industrial enzyme which is widely used in food, detergent, paper and textile industry. The significance of the study is to allow the enzyme for industrial usage by improving the stability properties. In order to determine the optimum conditions of immobilization, various parameters such as pH, incubation time and enzyme concentration were investigated. The immobilized enzyme exhibited better immobilization yield of 80%. Compared to free enzyme, the immobilized enzyme showed broader pH tolerance and temperature stability. Thermal stability study showed that there is considerable enhancement in stability of immobilized enzyme compared to free enzyme. The kinetic parameters, Km and Vmax were determined from Line weaver-Burk plot. The Km value for starch hydrolysis is found to be higher for immobilized enzyme than that of free enzyme and the Vmax value of free enzyme decreased moderately after immobilization. Storage stability study demonstrated that the immobilized enzyme preserved about 64.5% of its initial activity after six months of incubation. The frequent use experiment exhibited that the immobilized enzyme retained about 63% of its initial activity even after 10 cycles of reuses.

Keywords: Nanocomposite; Chitosan; TiO_2; Adsorption Immobilization; Maltose; Catalytic Activity;

Introduction

Enzymes are very efficient catalysts for biochemical reactions by providing an alternative reaction pathway of lower activation energy. Enzymes have worthwhile industrial and medical applications. The fermenting of wine, curdling of cheese, leavening of bread and brewing of beer have been practiced from earliest times, but not known these reactions to be as a result of catalytic activity of enzymes until the 19th century. Since then, enzymes have assumed an increasing importance in industrial processes that involve organic chemical reactions. Because of the low stability, high cost and high selectivity towards the reaction conditions, they are limited to industrial applications [1-3]. In order to facilitate their usage in industrial applications several developments have been done in the field of enzyme technology during the last two decades [4]. Immobilization of enzymes on solid supports has become most familiar technique after Nelson and Griffin have done the immobilization of invertase onto charcoal hydrolyses sucrose via adsorption in 1961 [5]. Immobilization provides many advantages, such as continuous operations in enzyme reactor, enhanced stability, reusability and easy separation from reaction mixture [6]. The introduction of immobilized catalysts has greatly improved both the technical performance of the industrial processes and their economy.

The methods used in immobilization are adsorption, covalent binding, and entrapment within a porous matrix, microencapsulation and aggregation [7,8]. Among these adsorption is the most important, easily operable method under moderate conditions and so it is the most economical way for enzyme immobilization [9,10]. In adsorption immobilization there is physical interactions generated between the carrier and enzyme that include van der Waals forces, ionic interactions and hydrogen bonding [11]. As the binding forces are too weak, it does not change the native structure of the enzyme and so prevents the distortion of active sites of the enzyme. Hence as a result of adsorption immobilization enzyme will retain its activity [12].

Another factor considered to be for enzyme immobilization is the selection of carrier [13,14]. They are classified as inorganic supports, synthetic polymers and natural macromolecules [15]. Polymeric carriers have the advantageous of good mechanical properties, ease of preparation, and applicability to introduce bio-friendly components for improving biocompatibility [16,17]. In the recent past, nanosized materials have been widely employed for enzyme immobilization. Due to the large surface

area, they provide superior loading capacity and low mass transfer resistance [18,19]. The usage of metal oxides for enzyme immobilization is now attracting too much attention. There is a report on successful enzyme immobilization on titanium (IV), iron (III), zirconium (IV), vanadium (III) and tin (IV) oxides [20]. Among this titanium (IV) oxide has been attracting too much attention because of the properties such as semiconductivity, biocompatibility, cost-effectivity and stability [21,22].

Chitosan, poly [β-(1→4)-2-amino-2-deoxy-D-glucose], is the biopolymer which contains amino and hydroxyl functional groups. It has many important chemical and biological properties such as biocompatibility, biodegradability bioactivity, nontoxicity and antibacterial properties [23,24]. The thermal, mechanical, chemical and physical properties of the polymer matrixes can be improved by addition of certain amount of inorganic oxides into polymer matrix [9]. In our study we have selected TiO_2 to form the nanocomposite with chitosan matrix so that the properties of the polymer can be improved. This nanocomposite is employed as the carrier for enzyme immobilization and seems to improve the performance of immobilized enzyme system.

The enzyme, α-amylase was applied as a model enzyme for immobilization. α-amylase is one of the largest selling enzymes for a wide variety of industrial applications. They are endo acting glycoside hydrolases which hydrolyzes α-1,4-glycosidic bond present in polysaccharides to produce glucose, maltose and maltotriose units [25]. These low molecular weight products are widely applied in the food, paper, textile, baking and brewing industries [26]. There are various types of supports have been previously investigated for the effective immobilization of α-amylase [27-32].

The current work deals with the immobilization of α-amylase onto chitosan- TiO_2 nanocomposite. The nanocomposite was synthesized by sol-gel method and characterized by IR, TGA, XRD and SEM analysis. We have done the immobilization of α-amylase onto the nanocomposite by adsorption. The immobilization parameters such as pH, contact time and amount of enzyme required for immobilization were optimized so as to acquire maximum immobilization yield and efficiency. The properties of the immobilized enzyme systems such as pH stability, temperature stability, thermal stability, reusability and kinetic parameters were compared with soluble enzyme. A novel biochemical method based on the detection of maltose using 3, 5-Dinitrosalicylic acid (DNS) method was employed to check the activity of the immobilized α-amylase. The results revealed that chitosan-magnetite nanocomposite is the promising carrier for enzyme immobilization.

Experimental

Materials

Diastase **α**-amylase (1, 4 α-D-glucanglucanohydrolase, EC 3.2.1.1) was purchased from Himedia Laboratories Pvt Ltd, Mumbai. Soluble Starch (potato) was acquired from S.D. Fine-Chem. Ltd, Mumbai. Chitosan was purchased from Meron marine chemicals, Cochin. All other chemicals were used analytical grade.

Amylase activity assay

Starch was selected as the substrate for determination of amylase activity. The reaction mixture consists of 1 mL of free α-amylase (0.5 mg/mL) and 1mL of 1% starch solution in desired buffer. The reaction system was incubated in a water bath with constant shaking at 30°C for exactly 15 min. The reaction was stopped by adding 1 mL 3, 5-dinitrosalicylic acid reagent. Incubation was performed in a boiling water bath for 5 min and cooling the reaction tubes to room temperature. The amount of reduced sugar (maltose) produced was determined spectrophotometrically at 540 nm [30]. An enzyme activity unit (EU) was defined as the amount of enzyme liberating 1 μmol maltose per minute under the assay conditions.

Total protein assay

The amount of immobilized α-amylase was measured by Lowry's method [33]. After the immobilization procedure the supernatant and washings were subjected to protein estimation using Folin Ciocaltaue's reagent by measuring the absorption at 660nm in a Thermo Scientific Evolution 201 UV-Visible spectrophotometer.

Synthesis of nanocomposite

Synthesis of TiO_2 Nanoparticles

TiO_2 nanoparticles were synthesized via sol-gel method on the basis of already reported work [34]. In the present study we have used methanol as solvent for synthesis and H_2SO_4 as the catalyst. Titanium tetraisopropoxide was dissolved in methanol keeping the molar ratio, TTIP: MeOH=1:3. Then distilled water was added drop wise into the solution in terms of a molar ratio of TTIP: H_2O = 1:4. H_2SO_4 was added to maintain acidic pH for restrain the hydrolysis process of the solution. The solution is then subjected to magnetic stirring for 40 minutes at room temperature. After that the gel formed is dried under 50°C for 1.5 hour to evaporate water and organic material to the maximum extent. Then it is undergone calcinations at 400°C for 2 h to obtain desired TiO_2 nanoparticles.

Synthesis of chitosan- TiO_2 (CST) nanocomposite

About 1 g nano TiO_2 powder was dissolved in 1% chitosan solution in acetic acid (1% v/v) by sonication. The solution stirred continuously until clear sol was obtained and after that 1M NaOH solution was added drop wise till the pH of solution became 10. The precipitate formed was heated at 80°C for 5 hour. Then the precipitate was filtered, washed with excess of water and dried in a vacuum oven at 60°C overnight [35].

Preparation of immobilized enzyme system

Immobilized enzyme was prepared by stirring 1g nanocomposite in enzyme solution in buffer at room temperature. The immobilization parameters optimized were pH (5-9), time (30-150 min) and the enzyme concentration (2 to 20 mg enzyme g^{-1} support). The biocatalyst then filtered and washed with the same buffer solution. The supernatant and washings were

subjected to protein estimation and the prepared immobilized enzyme was kept in a refrigerator at 4°C for further studies.

Immobilization yield (IY) was calculated by Eq. (1)

$$IY(\%) = \frac{c_1 - c_2}{c_1} \times 100 \qquad (1)$$

Where C1 is the concentration of protein introduced for immobilization and C2 is concentration of protein present in the supernatant after immobilization.

The activity yield (AY) was determined by Eq. (2)

$$\text{Activity Yield AY}(\%) = \frac{\text{Activity of immobilized enzyme}}{\text{Activity of free enzyme}} \qquad (2)$$

Immobilization efficiency was calculated using Eq. (3).

$$IE\ (\%) = \frac{AY}{IY} \times 100 \qquad (3)$$

Characterization of CST nanocomposite

The nanocomposite was characterized by using well-known physico-chemical methods. Surface groups and chemical structure were determined by FT-IR spectrometry using JASCO FT/IR-4100. Surface morphology of the nanocomposite was investigated by using the JEOL Model JSM –6390LV scanning electron microscope. Bruker AXS D8 Advance is used for XRD analysis. Thermal behavior of the nanocomposite was evaluated by using Perkin Elmer, Diamond TG/DTA.

Characterization of immobilized enzyme system

Optimum Temperature and Optimum pH

The optimum pH for maximum activity of free and immobilized enzyme was assayed by incubating the enzyme with starch over a pH range 5- 9 at 30^0C. The temperature for maximum activity was also assayed by varying the temperature from 30- 60^0C.

Thermal Stability

Thermal stability of free and immobilized enzyme was carried out by subjecting them to various temperatures ranging from 30-70°C for 1hour in a water bath. After 1hour of pre-incubation both free and immobilized enzyme in buffer cooled to optimum temperature and the enzymatic reaction was performed for a fixed time interval by adding definite amount of 1% starch solution to each reaction medium. Thermal inactivation curves with respect to incubation time for both the free and immobilized enzyme were obtained by pre-incubating them at their optimum pH and temperature. After definite time intervals, the enzymatic reaction was carried out and then the enzyme activity was calculated.

Reusability

The reusability of the immobilized enzyme was determined by repeated batch experiments maintaining a couple hours in each cycle. The residual activity of immobilized enzymes at its optimum conditions was measured at fixed time intervals. At the end of each cycle the immobilized enzyme was removed, washed with buffer solution and the reaction medium was changed with fresh substrate solution. The assay was carried out repeatedly 10 cycles under standard assay conditions.

Storage stability

The storage stability of immobilized enzyme was studied by evaluating its activity after being stored at 4°C in buffer solution for 6 months. The assay was conducted at regular intervals of time. The activity was compared with initial activity and was represented as percentage relative activity.

Kinetic Parameters

To determine the kinetic parameters, Michaelis constant (Km) and maximum rate (Vmax) for free and immobilized enzyme, the enzymatic assays were carried out by varying the substrate concentrations ranging from 0.2 to 1.0 mg/mL at optimum temperature and pH. The parameters were calculated from the Lineweaver - Burk plot.

Results and Discussion

Characterization studies

The IR spectra of chitosan nanoparticle, TiO_2 nanoparticle and chitosan-TiO_2 (CST) nanocomposite were shown in the supplementary data (Appendix A). In case of chitosan nanoparticle and CST nanocomposite, the absorption band in the region of 3750-3000 cm^{-1} was attributed to the combined peaks of amino (-NH_2) and hydroxyl (-OH) groups stretching vibration. This band become much broaden in case of CST nanocomposite which may arise from the hydroxyl group that belongs to linear polymeric chain of $TiO(OH)_2$. The IR spectra of TiO_2 nanoparticle showed the peaks at 3400 and 1620 cm^{-1}due to the stretching and bending vibration of the -OH group. Also the band at 520 cm^{-1} showed stretching vibration of Ti-O and at 1450 cm-1 showed stretching vibrations of Ti-O-Ti. The Ti–O band in the range of 400–700cm^{-1} can be seen in case of CST nanocomposite which was ascribed to the composite formation of TiO_2 with chitosan. The peaks related to CH_2 asymmetric vibration could be seen at 2925, 2870, 1430, 1320 and 1250 cm^{-1} [9]. The absorption band at 1074 cm^{-1} represented the C-O stretching vibration of primary alcoholic group in chitosan. The peak observed at 1585 cm^{-1} corresponding to amide II and the two peaks at 1655 and 1320 cm^{-1} representing the amides I and III [36]. Increased intensity of amide II peak in the spectrum of CST nanocomposite was observed which may be resulted from the interaction of Ti^{+4} with the amide group of chitosan.

SEM analysis was performed in order to observe surface topography and morphology. From the SEM micrographs of TiO_2 nanoparticle and CST nanocomposite (supplementary data-Appendix B), it is clear that the nanocomposite has aggregated particle structure. The image reveals the surface structure of nanocomposite with small-flake like surface presented separately in the exterior morphology. This aggregated and agglomerated morphology of the CST nanocomposite confirms the dispersion of TiO_2 nanoparticles into the chitosan matrix and also the nanocomposite formation.

The TGA analysis has done under nitrogen atmosphere at a heating rate of 10^0C/min in the temperature range of 30–730^0C. The TG curves of chitosan nanoparticles and CST nanocomposite (supplementary data- Appendix C) exhibit almost similar thermal degradation trend. Two stages of weight loss can be seen for both the samples. The first stage of weight loss, from room temperature to 200 °C could be as a result of the evaporation of adsorbed water. Nearly 10% weight loss was observed in the first step. The major weight loss was observed in second step between 250 and 500°C. It was reported that this weight loss was caused by decomposition of the polysaccharide and loss of (O–H) groups of TiO_2 dispersed in polymer matrices [37]. After 500°C there is no significant weight loss was observed. At the end of the analysis, about 60% of weight loss for chitosan nanoparticle and 50% of weight loss for CST nanocomposite were observed at 700^0C.

XRD diffraction patterns of TiO_2 nanoparticle and CST nanocomposite are shown (supplementary data- Appendix D). Diffraction peaks in XRD pattern of nanoTiO_2 are sharper and stronger at 26.9°, 36.5°, 39°, 44°, 54° and 55.5°. All the diffraction peaks are also seen in case of CST nanocomposite. This revealed that the CST nanocomposite is successfully formed. The TiO_2 has the crystal structure assigned to anatase-type titanium (JCPDS 21-1272). Anatase structure of the TiO_2 crystals was preserved even after the formation of nanocomposite. The peaks at 2θ= 5° and 25° were characteristic peaks corresponding to chitosan.

The average crystalline size can be quantitatively evaluated from the XRD data using the Debye–Scherrer equation which gives a relationship between peak broadening in XRD and particle size: $d = (k\lambda/\beta\cos\theta)$.

Where d is the particles size, k is the Debye–Scherrer constant (0.89), λ is the X-ray wavelength (0.15406 nm) and β is the full width at half maximum, θ is the Bragg angle. The most intense diffraction peak at 26.9^0 attributed to anatase phase of TiO_2 can be seen for TiO_2 nanoparticle and CST nanocomposite. According to the Debye-Scherrer equation, the crystalline size of the TiO_2 nanoparticle is 26.75nm and that of CST nanocomposite is 28.32nm. This data indicates that the synthesis of CST nanocomposite did not much affect the size of TiO_2 nanoparticle [36].

Optimization of immobilization parameters

The parameters affecting immobilization of enzyme such as pH of the immobilization medium, incubation time and the amount of enzyme were optimized. The retained activity of the immobilized enzyme was designated in terms of relative activity. Figure 1a exhibited the effect of pH on immobilization. The optimum pH for the immobilized enzyme, CSTE was at pH 6. The isoelectric point of α-amylase is around 4.6 and the amino group in chitosan has a pKa value of about 6.5. At the optimum pH 6 there is significant electrostatic interaction between the support and the enzyme since chitosan having net positive charge and the enzyme molecule having net negative charge. The decrease in activity above and below this pH may be due to lower adsorption which occurred as a result of unfavorable charge distribution on enzyme and the support.

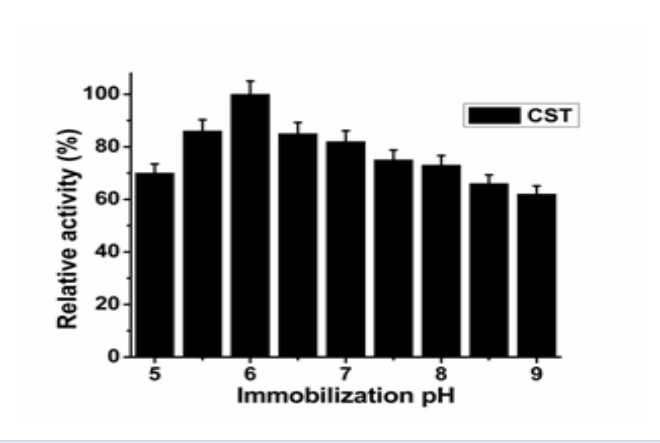

Figure 1a: Effect of pH of immobilization medium on the relative activity of immobilized α-amylase.

Figure 1b showed the effect of adsorption time on the relative activity of the immobilized enzyme. The adsorption time was varied in the range between 30 and 150 min. and the other parameters kept constant. The enzyme activity increased up to 90 min. and after this point, a decrease in the activity of immobilized enzyme was observed. The decrease in activity could be as a result of multilayer adsorption of enzyme on the surface of support which deformed the active site of the enzyme.

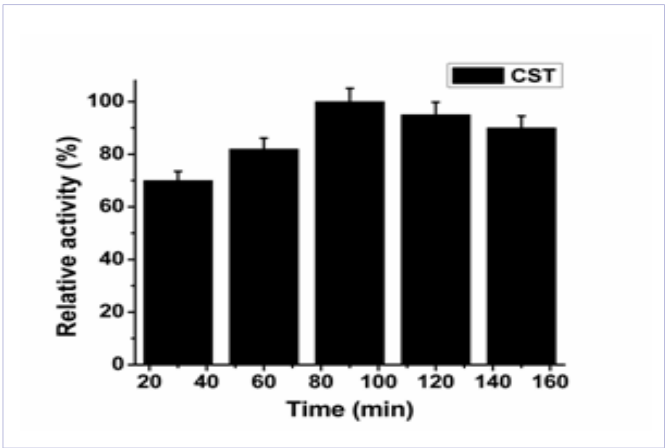

Figure 1b: Effect of contact time on immobilized enzyme activity.

The amount of enzyme adsorbed and the activity of immobilized enzyme were determined. As the enzyme concentration increased, the amount of enzyme adsorbed on the support increased and then reached a saturation point. It was depicted in the figure 1c. The effect of enzyme concentration on activity also exhibited in the figure 1d and the decrease in the activity after the saturation point is due to conformational changes of the enzyme or the steric hindrance caused by the support at the active site [38]. The immobilization yield, activity yield and immobilization efficiency were calculated and the results are given in the table1.

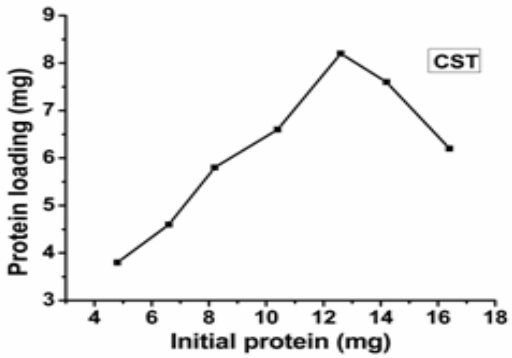

Figure 1c: Effect of initial protein amount on protein loading.

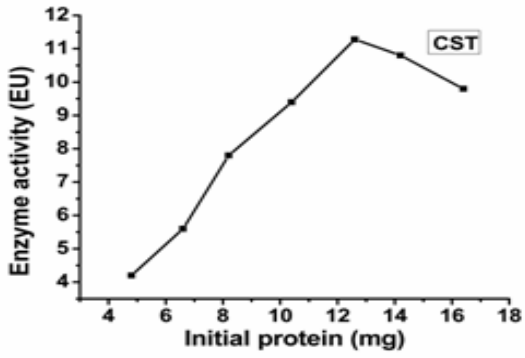

Figure 1d: Effect of initial protein concentration on immobilized enzyme activity.

Table 1: Immobilization efficiency of CST nanocomposite

Support	Initial protein (mg)	Immobilized protein mg/g support	IY (%)	Initial activity (EU)	Immobilized enzyme activity (EU)	AY (%)	IE (%)
CST	12.6	8.2	65.08	20.56	11.28	54.86	84.30

Parameters affecting enzyme activity

Effect of pH on enzyme activity

We have investigated the effect of pH on the activities of free and immobilized α-amylase and the results are shown in the figure 2a. The relative activity of immobilized enzyme, CSTE was higher than that of free enzyme. Chitosan did not show any acidic shift due to its solubility in lower pH range. In the present study, interaction of Ti^{+4} with the $-NH_2$ group of chitosan caused the decrease of solubility of chitosan matrix in acidic media. The positively charged TiO_2 surface was keeping away the H^+ ions from the microenvironment of enzyme and the activity of immobilized enzyme, CSTE could be protected in lower pH range [39]. It was found that the free enzyme showed its maximum activity in the range of pH 5–5.5. The optimum pH of immobilized enzyme, CSTE was at pH 6, representing 0.5 units increase when compared to free enzyme. This result agreed to Egwim et al. [40] reported that the optimum pH of free lipase was 6.5, while that of immobilized lipase was at pH 7 (a 0.5 unit increase). The shift of optimum pH of the enzyme to alkaline region could be explained by poly cationic nature of chitosan. They attract more OH^- ions making the microenvironment of enzyme more alkaline than the bulk solution and leads to a shift in pH towards basic region.

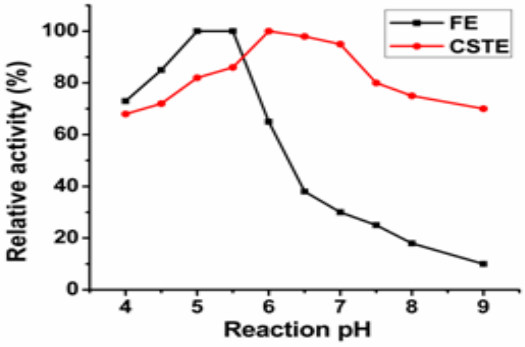

Figure 2a: Effect of pH on the activity of free enzyme and CSTE.

Effect of temperature on the enzyme activity

The temperature effect on activity of free and immobilized enzyme was shown in the figure 2b. It was found that the relative activity of immobilized enzyme was higher than that of free enzyme. This result indicate that adsorption of enzyme on nanocomposite limited the movement of enzyme and increased the conformational integrity. The addition of TiO_2 nanoparticles into the chitosan matrix increased the temperature characteristics and decreased the diffusional restriction of product and substrate

at higher temperature. This led to the increased activity of the immobilized enzyme system. The free α- amylase exhibited the optimum temperature of 50°C and this value shifted to 35°C for the immobilized enzyme, CSTE. The decrease of the optimum temperature may arise from the change of the conformational integrity of the enzyme structure through the interactions with the nanocomposite which resulted in an alteration of enzyme substrate affinity. The decrease of optimum temperature of α-amylase was reported when immobilized on silica gel [41].

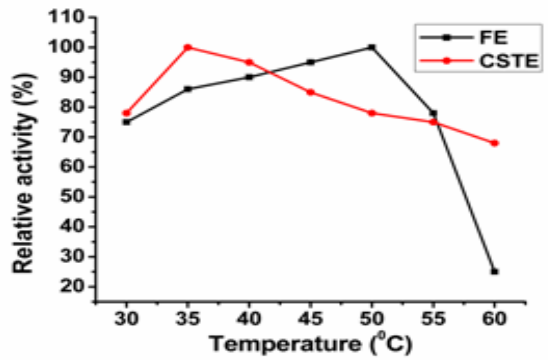

Figure 2b: Effect of temperature on activity of free enzyme and CSTE.

Thermal stability

The immobilized enzyme with higher thermal stability is beneficial for different industrial applications. This property of free and immobilized enzyme was investigated in the range of 30–70^{0}C to assess the potential advantage and the results are shown in figure 3a. After 1 hr pre-incubation at 50^{0}C, the free enzyme lost almost 60% of its initial activity and that of immobilized enzyme was 15%. At 70^{0}C the free enzyme retained 10% of its initial activity, whereas immobilized enzyme retained above 70%. The immobilized enzyme, CSTE exhibited better thermal stability since its activity decreased slower rate as the temperature increased to higher region. It preserves the tertiary structure of the enzyme and protects the enzyme from conformational changes of the active center caused at higher temperatures [9].

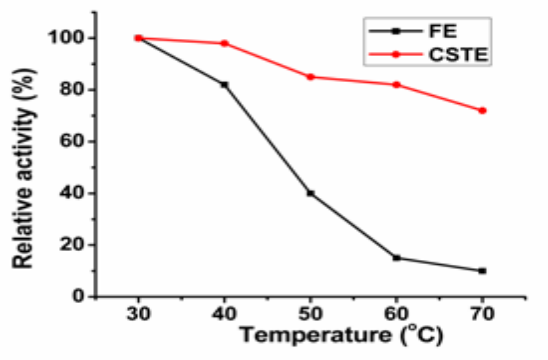

Figure 3a: Thermal stability of free enzyme and CSTE pre-incubated for 1h at different temperatures

The thermal stability of free and immobilized α-amylase with respect to pre-incubation time was studied and the result was depicted in figure 3b. The figure showed that immobilized α-amylase retained more than 90% activity even after 120 min of pre-incubation at optimum temperature. This indicates that the relative activity of the immobilized enzyme was decreased slowly compared to that of free enzyme. The slow decrease in activity of immobilized enzyme was due to its restricted mobility which preserved the three dimensional conformation of enzyme from denaturation. The increase in thermal stability of α-amylase was observed when immobilized on nano ZnO [42].

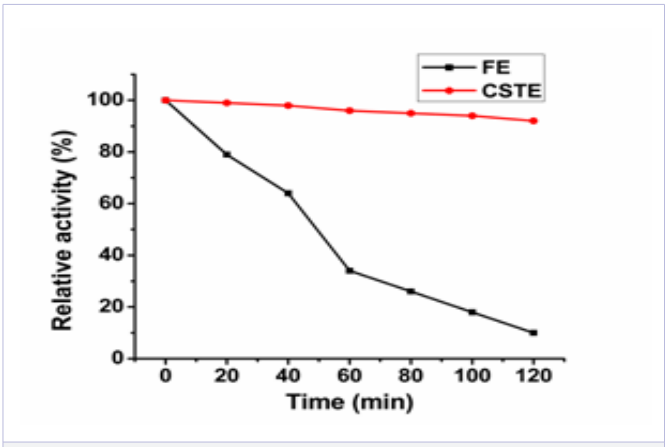

Figure 3b: Effect of pre-incubation time on activity of free enzyme and CSTE at their optimum temperature.

Kinetic parameters

The kinetic parameters, Michaelis–Menten constant (Km) and maximum reaction velocity (Vmax), for free and immobilized α-amylase were determined using Lineweaver–Burk plot. These parameters were estimated using starch as the substrate. Vmax indicates the intrinsic characteristics of the enzyme which gives the maximum reaction velocity and Km is a measure of the substrate's affinity for the enzyme. Km can change due to the conformational changes of the enzyme molecule. Higher values of Km indicate lower substrate affinity for the enzyme [43] as the shown in the table 2, the Km value for the immobilized enzyme (0.58 mg/ mL) was higher than that of the free enzyme (0.45mg/ mL).

Table 2: Kinetic parameters of the free and immobilized α-amylase.

	Free enzyme	CST
K_m(mg/ml)	0.45± 0.02	0.58 ± 0.03
V_{max}(μmol mg^{-1} min^{-1})	34.48±0.05	24.39±0.04

The increase in Km value for immobilized enzyme was due to its conformational changes, resulting in a lower affinity for the substrate compared to the free enzyme. The increase in Km value for immobilized α-amylase was also reported by M.A. Abdel Naby et al. [44]. Vmax values were found 34.48 and 24.39 μmol mg^{-1} min^{-1} for free and immobilized enzyme respectively. The decrease in Vmax value represents that the action of the enzyme immobilization reduced the enzyme activity [45]. This reduction may be caused due to the lower accessibility of the substrate to the active site of immobilized enzyme. The structural changes in the enzyme as a result of immobilization may also lead to change in affinity to its substrate [46].

Effect of frequent use on activity of immobilized enzymes

The frequent use of immobilized enzyme is the key factor for its cost effective employability in industrial applications. We evaluated the reusability of the immobilized enzyme, CSTE keeping a couple hours in each cycle. CSTE can be reused either by centrifugation or filtration and it retained nearly 63% of initial activity after ten reuses (Figure 4a). The subsequent decrease in activity after every cycle could be due to desorption, denaturation or conformational changes of the enzyme molecule upon uses. The reusability of immobilized α-amylase was investigated in several reports [47-49].

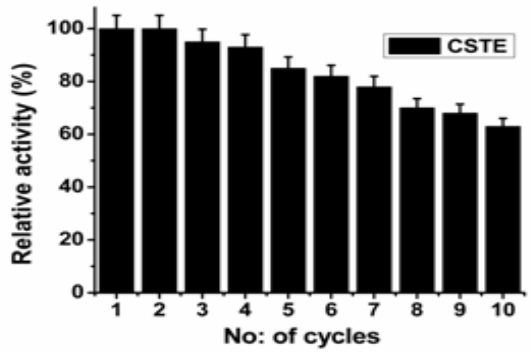

Figure 4a: Reusability of CSTE

Effect of storage time on activity of immobilized enzymes

The free enzyme in solution is not stable during storage and the activity gradually reduced. The storage stability of immobilized enzyme is the important factor in order to ensure its long shelf life. In our study, immobilized enzyme was stored in buffer at 4^0C and the activities were measured after definite periods of storage. The immobilized enzyme retained 64.5% of its activity after 6 months and the results are depicted in the figure 4b. The study showed that immobilized enzyme gained more stable character than the free one. The ionic interactions generated between enzyme and the carrier imparted a higher conformational stability to the immobilized enzyme. Many reports are there showing the storage stability of immobilized α-amylase [50-52].

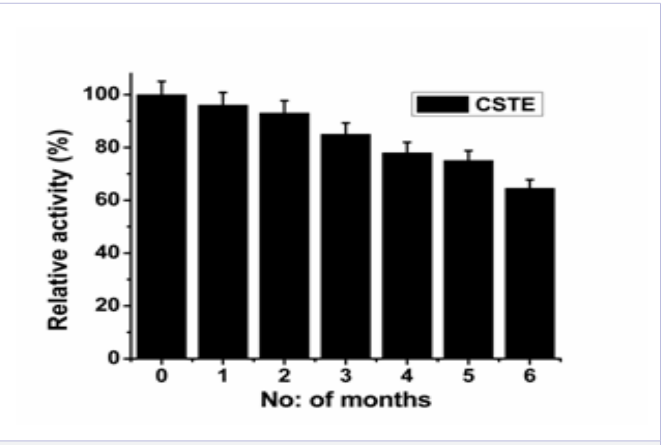

Figure 4b: Storage stability of CSTE

Conclusion

In this work, α-amylase was successfully immobilized onto chitosan-TiO_2 nanocomposite by adsorption. The method used for immobilization was simple and economical. We have optimized the immobilization parameters such as pH, incubation time and enzyme concentration. We improved the stability properties of α-amylase by using the immobilized enzyme system. Enzyme assays demonstrated that substantial improvement in thermal, pH and storage stability. The immobilized enzyme showed excellent reuse potential maintaining high levels of activity for repeated use without much affecting the integrity of the catalytic sites of the enzyme. The kinetic study showed that the substrate affinity for the immobilized enzyme in enzymatic reaction is less and exhibits higher Km and lower Vmax compared to the free enzyme, indicate decreased activity. The results represented the potential applicability of the immobilized enzyme for industrial applications, which could be used for the efficient starch hydrolysis.

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