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# Immobilization of β-Galactosidase from *Escherichia coli* onto Modified Natural Silk Fibers

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**ABSTRACT:** A polymeric support based on the natural silk fibers was prepared and characterized for covalent immobilization of β-galactosidase from *Escherichia coli*. The silk fibers were grafted using polyacrylonitrile in presence of benzophenone as a photo-initiator. The grafted fibers were then activated by treatment with hydrazine hydrate followed by glyoxal cross-linker. FTIR spectra, scanning electron microscope (SEM) in addition to the staining test derived from the Coomassie protein assay were utilized for investigation of the modification and immobilization steps. Also, the activity of both free and immobilized β-galactosidase was evaluated as a function of the various important parameters like grafting percentage, pH, and temperature. In addition, the kinetic parameters $K_m$ and $v_{max}$ for both free and immobilized enzyme were anticipated using Michaelis–Menten equation. The results in this study indicated that the prepared modified woven silk fibers could be used effectively as a polymeric support for immobilization of β-galactosidase. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 2923–2931, 2013

**KEYWORDS:** bioengineering; biomaterials; fibers; grafting

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## INTRODUCTION

Various types of living microorganisms like plants, bacteria, and fungi are known to be essential sources of the β-galactosidases enzymes, which are utilized in numerous applications in food industry such as improvement of the sweetness, flavor, and edibleness of dairy food.[1] Breakdown of the main milk sugar disaccharide lactose into the monosaccharide glucose and galactose considered as the key utilization of β-galactosidase. Many people struggling with lactose intolerance cannot consume milk in its native form and subsequently hydrolysis of lactose makes milk suitable to feed such people.[2] Transgalactosylation is another important reaction utilizing β-galactosidase in which the enzyme will convert galactose to galactose or glucose hydroxyl groups during the hydrolysis of lactose yielding galacto-oligosaccharide (GOS).[3,4] GOS are recognized as non-digestible carbohydrates that known to be prebiotics thus enhance the growth of Bifidobacteria within the lower part of the human intestine.[5,6]

The application of the free enzymes in industrial processes is somehow restricted as a result of high price, recovery problem following the reaction completion and instability. Subsequently, the enzymes utility in their immobilized forms provides a numerous benefits when compared to their free forms for examples, the convenience of recovery from the reaction mixture once the enzymatic process completed and reusability, which needless to say add an economic value through the cost reduction, process control improvement, and stability increase. On the opposite hand, immobilization typically causes some changes regarding the kinetic parameters of the enzymes as a result of the restricted movement of the protein macromolecule once fixed on the support.[7–9] Many significant parameters like reactivity, efficiency, stability, and accessibility to the support ought to be considered when choosing a carrier support for the immobilization process.[8,10,11]

Among various types of materials that can be used as a support carriers for enzyme immobilization, fibers like materials whether derived from natural source such as cellulosic cotton and keratinous protein fibers or artificial fibers such as polyamide nylon, acrylic, and polyester fibers, have been implemented as a polymeric carrier supports for immobilization of enzyme, this may be due to their superb properties compared to the other types of immobilization supports, like the relatively high surface area, strong mechanical properties, resistance toward chemicals, and sometimes biological activity.[12–17] Protein worm silk fiber is one of those fibrous polymeric materials, which exhibits an excellent attainable within the field of enzyme immobilization,[18] this could be attributed to both the very good physicochemical behavior of the protein backbone, which has the capacity to keep consistently the biological activity of the bonded protein enzyme macromolecule and high

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accessibility of various reactive sites containing different functional carboxylic and amino groups from the amino acid residues, which enhance the surface modification to facilitate the fixation of the enzyme. Furthermore, the smooth regular homogeneous silk fibers may contribute in lowering both substrate and product diffusional limitations observed in porous support materials. Finally, beside all the aforementioned advantages, the silk fibers are cheap, highly accessible, and non-toxic which makes it utilized in immobilization of several types of enzymes like alkaline phosphatase,[19] uricase,[20] glucose oxidase,[21] and lipase [22].

Here, in this study, a brand new fibrous polymeric support based on natural worm silk fibers was prepared by means of graft copolymerization of polyacrylonitrile (PAN) using benzophenone as a photo-initiator. The grafted fibers were then activated by hydrazine hydrate followed by glyoxal cross-linker to be utilized in immobilization of β-galactosidases enzyme. Various instrumental techniques such as scanning electron microscope and FTIR spectra were applied to confirm the structure of the fibers before and after modifications and β-galactosidases immobilization. Also, the influence of numerous vital parameters like degree of grafting, pH, and temperature on the activities of both free and immobilized enzyme was investigated.

## EXPERIMENTAL

### Materials

Natural silk fibers has been collected from the farm of the Manoura high agriculture school and handled based on the popular degumming and spinning techniques.[23] The acrylonitrile (AN) monomer (Aldrich) was purified to get rid of the stabilizer by treatment with 3% NaOH solution then washed with distilled water until alkali free and after all stored over molecular sieves. Benzophenone, hydrazine hydrate 98%, glyoxal, β-Galactosidase from *Escherichia coli* with specific activity 200 U $mg^{-1}$, 2-nitrophenyl-β-D-galactopyranoside (ONPG) 98%, hydroquinone 98%, and sodium carbonate 99% were purchased from Sigma-Aldrich and used as supplied.

### Grafting of PAN onto Silk Fibers

The details of the materials and quantities used throughout the photo-induced graft copolymerization of PAN onto the natural silk fibers are summarized in Table I. In a Pyrex tube, the appropriate amounts of silk fibers soaked in the photo-initiator benzophenone solution in methanol and the mixture was irradiated with ultraviolet light using 450 W high pressure mercury lamp (USH-450GL) under $N_2$ atmosphere. Ten minutes later, the needed amount of the monomer (AN) was added and the reaction continued for another 1 h. Then, the reaction was terminated by adding 5 mL 3% hydroquinone solution and the fibers were removed and washed with DMF to extract the PAN homopolymer. Finally, the fibers were dried in an oven at 40○C until constant mass.

The grafting percentage was estimated using the following equation.[13]

$$\text{Grafting Percentage (GP)} = (A-B/B) \times 100 \quad (1)$$

Where $A$ is the mass of the grafted silk fibers and $B$ is the mass of the used silk fibers before grafting. The grafted fibers (silk-g-PAN) with GP values 35, 87, and 130% were named as $S_1$, $S_2$, and $S_3$ respectively.

**Table I.** Synthesis of S-*g*-PAN Under Various Conditions

| Formulation code | AN (mol $L^{-1}$) | GP (%) |
|---|---|---|
| $S_1$ | 0.95 | 35 |
| $S_2$ | 1.4 | 87 |
| $S_3$ | 2.3 | 130 |

Concentration of silk fibers = 5 g $L^{-1}$ ; Benzophenone = 3.5 × $10^{-2}$ mol $L^{-1}$ ; irradiation time was 1 h at room temperature.

### β-Galactosidase Immobilization

Two consecutive steps had been performed in an effort to activate the previously prepared silk-*g*-PAN for β-galactosidase immobilization. In the beginning, nearly all –CN groups belonging to the grafted PAN chains were turned into HN=C–$NHNH_2$ groups by refluxing a combination of 500 mg of the grafted fibers and 50 mL 20% v/v hydrazine hydrate solution in ethanol at 80○C for 4 h. The fibers were then removed and washed with ethanol and dried in oven for 5 h at 40○C. The acquired modified fibers had been named silk-*g*-PAH.

In the next modification phase, the modified silk-*g*-PAH fibers were soaked in 50 mL 20% glyoxal solution for 4 h aiming to activate the fibers. The activated fibers were then filtered and washed with distilled water. The β-galactosidase immobilization was finally carried out by soaking the active fibers in 14 mL aqueous β-galactosidase solution containing a variety of concentrations from 1 to 6 mg $mL^{-1}$ in phosphate buffer (50 mM, pH 6.5). The mixture used to be then vigorously shaken for 12 h at 5○C after which, the fibers containing the immobilized β-galactosidase were removed and washed with similar buffer solution. The artificial strategies for the fibers modification and immobilization were schematically introduced in Scheme 1.

The immobilization efficiency (IE%) and subsequently the amount of the immobilized protein enzyme had been anticipated using the following *eq.* (2).[24]

$$\text{IE}\,\% = \left(E_0 V_0 - E_f V_f / E_0 V_0\right) \times 100 \quad (2)$$

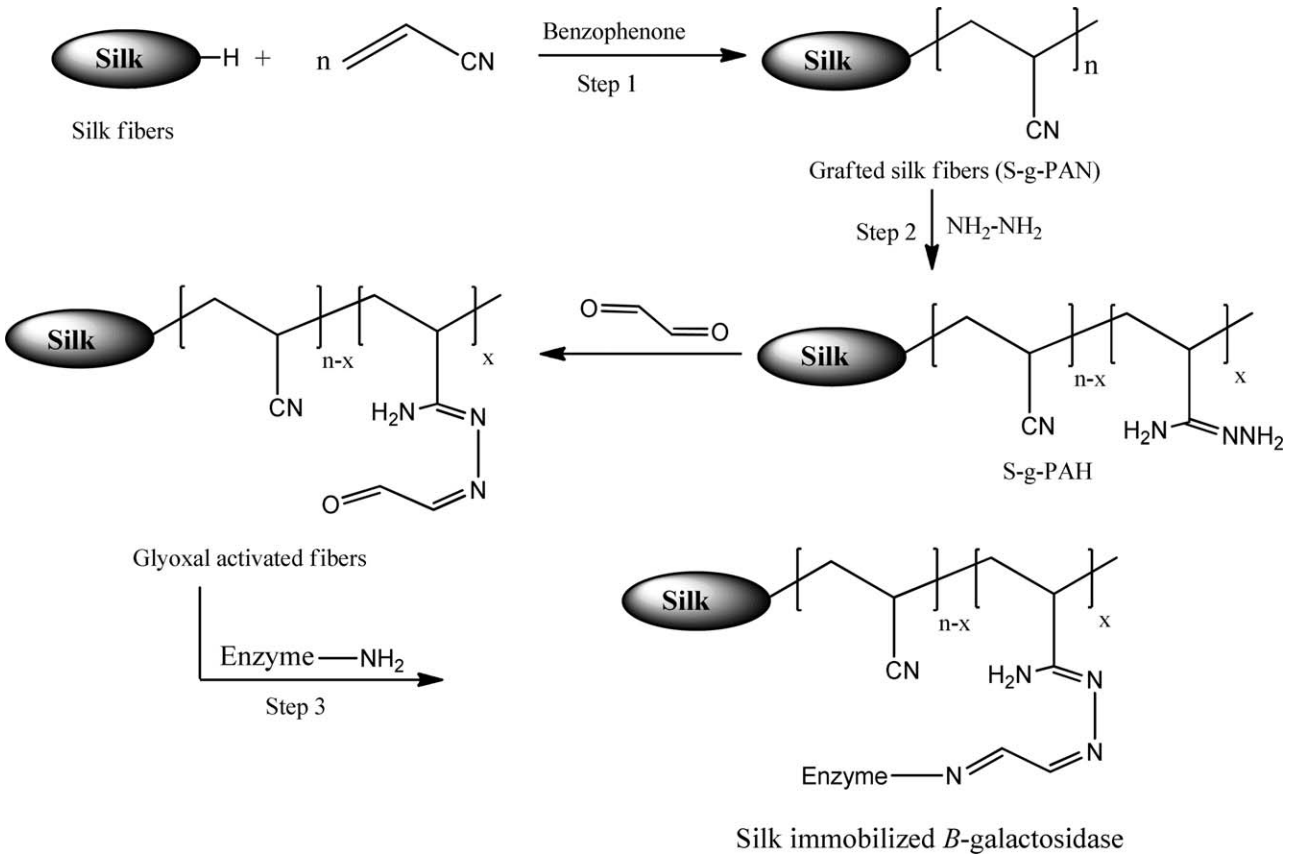
Where $E_0$ is the β-galactosidase initial activity (U $mL^{-1}$); $V_0$ is the initial volume of β-galactosidase solution (mL); $E_f$ is the β-galactosidase activity in the filtrate (U $mL^{-1}$); and $V_f$ is the filtrate volume (mL).

### IR-Spectroscopic Analysis

Perkin-Elmer 1430 Infrared spectrometer was utilized to investigate the chemical structures of both native and modified silk fibers before and after β-galactosidase immobilization using compressed KBr discs containing the polymeric samples of the samples with KBr to form.

### SEM Analysis

FEI Quanta-200 electron scanning microscope (USA) was applied to visualize the morphology of the fiber samples after




**Scheme 1.** Presentation of silk grafting, modification, and β-galactosidase.

coating with Au for 40 s at 15 mA. Accelerating voltage 10 kV was operated.

### Staining Test

Modified Coomassie protein assay[25] used to be utilized in order to ensure the cross-linking of the enzyme protein to the modified silk fibers. The dye solution was prepared via dissolving 100 mg of Coomassie Brilliant Blue G-250 in 100mL 85% (w/v) ethanolic phosphoric acid solution after which dilution of the mixture to 1000 mL. The prepared dye solution was filtered prior to use.

The staining used to be performed by soaking the fiber samples into the dye solution for 5 min followed by washing with distilled water.

### Assay of β-Galactosidase Activity

In accordance with earlier studies,[26,27] ONPG used to be implemented as a substrate to anticipate the β-galactosidase activity. A mixture of 5 mL phosphate buffer solution, 2.0 mM ONPG and the appropriate amount of the enzyme was allowed to react at 37○C for 10 min. After which, the reaction was finalized using sodium carbonate solution (2 mL, 1.0*M*) and the amount of the launched o-nitrophenol was estimated by measuring the absorbance at 405 nm. The β-galactosidase activity defined as in the following: one unit correspond to the amount of the enzyme that able to hydrolyze 1 μmol of ONPG $min^{-1}$. For the immobilized β-galactosidase samples, the activity was estimated by following the same procedure; however the fibers were withdrawn prior the reaction termination and measurements. The relative activity used to be calculated using *eq.* (3).

$$\text{Relative activity } (\%) = (\text{Activity} / \text{maximum activity}) \times 100 \quad (3)$$

### Kinetic Properties

The kinetic parameters such as Michael's constant value ($K_m$) and maximum rate ($v_{max}$) were evaluated by using a series of various concentrations of the substrate ONPG in phosphate buffer (50 mM, pH 6.5) at 37○C.

### Enzyme Stability and Reusability

In order to judge the thermal stability, both free and immobilized β-galactosidase samples were incubated at 50○C and pH 6.5. Every 1 h a test sample was withdrawn to measure the residual activity. For evaluating the reusability, six consecutive operating cycles were performed using another fresh β-galactosidase immobilized sample. After the completion of each cycle, the fibers were eliminated and stored for 24 h at 5○C and then reused with new ONPG substrate sample.

## RESULTS AND DISCUSSION

### Characterization of the Samples

β-Galactosidase enzyme was covalently immobilized on the modified grafted silk fibers after activation by glyoxal cross-linker.

The FTIR spectra were applied to hit upon the consecutive modification and immobilization steps which had been

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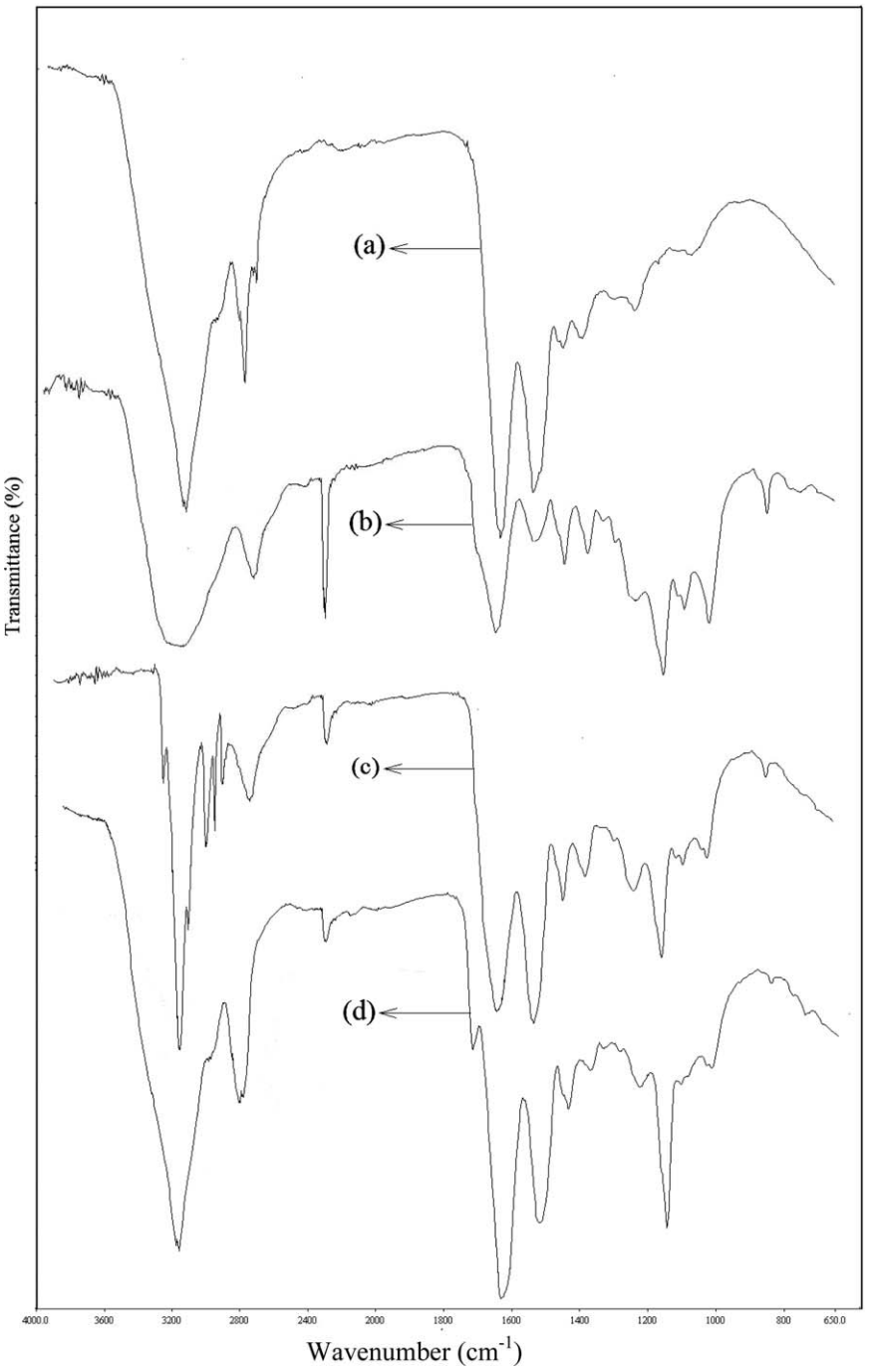


**Figure 1.** FTIR spectra of (a) native silk, (b) S-*g*-PAN ($S_2$), (c) $S_2$-*g*-PAH before *β*-galactosidase immobilization, and (d) $S_2$-*g*-PAH after *β*-galactosidase immobilization.

performed on the silk fibers. As can be noticed in Figure 1(a), the spectrum of pure native silk fibers exhibited a robust wide absorption band at approximately 3000 $cm^{-1}$, which could be attributed to the –OH and N–H bonds of the protein macromolecules of silk fibers. Additionally, the different polyamide diagnostic peaks between 600 and 1700 $cm^{-1}$ confirm the construction of the silk fibers which was in accordance with earlier reports.[28,29] The primary modification step in which the native silk fibers were grafted with PAN was clearly confirmed through the obvious appearance of the characteristic –CN beak at about 2350 $cm^{-1}$ and partial disappearance of the broad peak at 3000 $cm^{-1}$ [Figure 1(b)]. Alternatively, as presented in Figure 1(c), the further modification regarding the conversion of the vast majority of the grafted –CN groups into HN=C–NH–$NH_2$ was detected by the obvious disappearance of the feature –CN beak at 2350 $cm^{-1}$ and clear appearance of sharp peak at about 1630 $cm^{-1}$ belonging to the C=N in addition to clear duplet peak at about 3150 $cm^{-1}$ corresponding to the –NH–$NH_2$. After all, the fixation of the *β*-galactosidase protein macromolecules was also elucidated via re appearance of extensive broad band at about

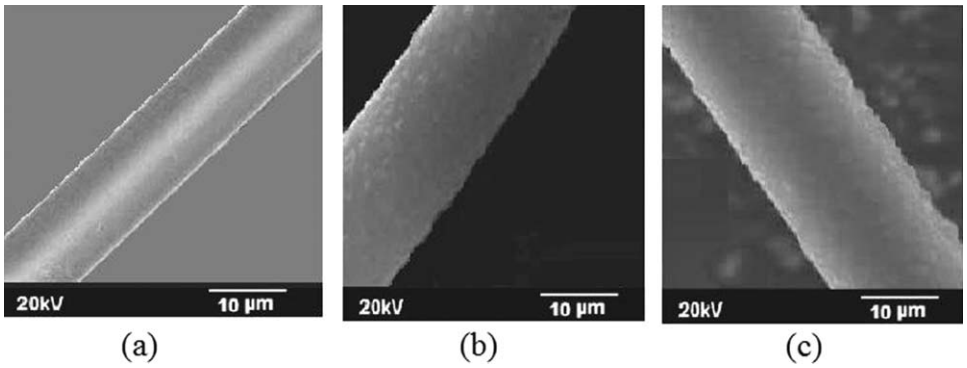


**Figure 2.** SEM photos of modified and unmodified silk fibers. (a) Pure native silk fibers, (b) S-*g*-PEA ($S_2$), and (c) S-*g* PAH after *β*-galactosidase immobilization.

3000–3500 $cm^{-1}$, which may attributed to the O–H and N–H bonds of the inserted enzyme protein, in addition to the obvious increase of the sharp peak at 1639 $cm^{-1}$, which may correspond to the C=O bond of the protein amide [Figure 1(d)]. These results may give an evidence for the covalent fixation of the *β*-galactosidase enzyme onto the modified silk fibers.

The morphology studies for both native and modified silk fibers earlier than and after the *β*-galactosidase fixation had been investigated using scanning electron microscope (Figure 2). As can be observed, the grafted silk fibers $S_2$ exhibited an evident diameter increase compared to the native fibers; this could be attributed to the coating with PAN grafted layer. Additionally, the *β*-Galactosidase immobilized fibers provide an increase in both diameter and roughness, which is anticipated due to the covalent fixation of the macromolecular enzyme protein onto the modified fibers.

For additional confirmation of *β*-galactosidase immobilization on the modified silk fibers, staining test using Coomassie Brilliant Blue G-250 dye was utilized for detection of the enzyme protein covalently fastened on the polymeric fiber support. As discussed in earlier reports,[30] the Coomassie Brilliant Blue G-250 dye able to interact with arginine amino acid residues generating blue colored dye-protein complex. In Figure 3, native silk fibers which are protein in nature provide a faint blue color [Figure 3(a)]. Then again, the color depth showed a vital decrease after PAN grafting [Figure 3(b)], which can be attributed to the insertion of the hydrophobic PAN chains and subsequent accessibility limitation of the dye molecules to the protein backbone. Also, the blue color depth decrease could be due to the lower percentage of the protein silk fibers in the PAN grafted copolymer compared to the native silk fibers. Alternatively, after the *β*-galactosidase immobilization, the color depth exhibited an obvious increase [Figure 3(c,d)], which confirms the presence of the enzyme protein macromolecules on the surface of the modified fibers.

### Immobilization of *β*-Galactosidase

Based on the calculations derived from *eq.* (2), the amount of the *β*-galactosidase immobilized onto the modified silk fibers $S_2$ in addition to the immobilization efficiency had been studied against the concentration of the *β*-galactosidase solution utilized during the immobilization procedure. In Figure 4, raising the *β*-galactosidase solution was accompanied by a corresponding increase in the *β*-galactosidase immobilized amount and a decrease in the immobilization efficiency. This can be explained as in the following: by raising the concentration of the used *β*-galactosidase solution, the macromolecular protein molecules of the enzyme will covalently attached to the active aldehyde units on the fibers until these units are completely saturated, then any excess will not cross-link to the fiber polymeric support and subsequently get lost during the washing step, which explain the lowering in the immobilization efficiency. The maximum *β*-galactosidase amount gets fixed on the $S_2$ fibers is 75.5 mg per 1 g fibers.

### Influence of *D*egree of *F*unctionalization on the *I*mmobilization *E*fficiency and *E*nzyme *A*ctivity

During the modification of the grafted silk fibers, the vast majority of the –CN groups turned into HN=C–NH–$NH_2$ groups

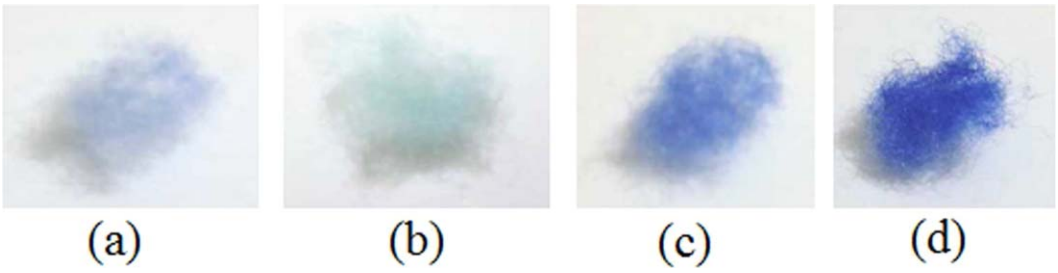


**Figure 3.** Digital photograph of silk fibers after dipped into Coomassie Brilliant Blue solution of 5 mL for 5 min: (a) Pure native silk fibers, (b) S-*g*-PAN ($S_2$) (c) S-*g*-PAH (from $S_2$) after *β*-galactosidase immobilization, and (d) S-*g*-PAH (from $S_3$) after *β*-galactosidase immobilization. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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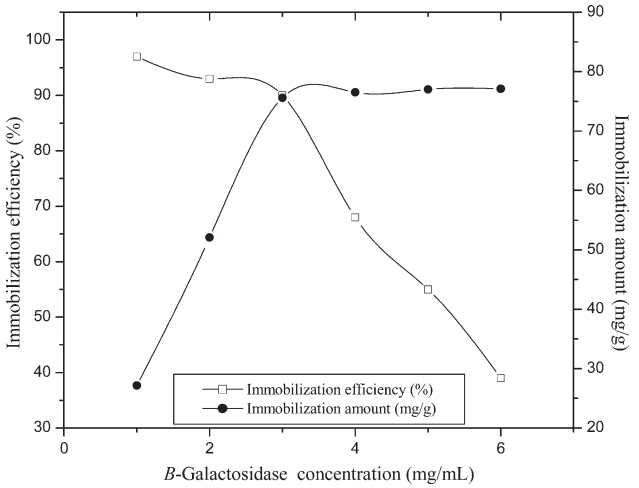


**Figure 4.** $\beta$-Galactosidase immobilization on the S-*g*-PAH (from $S_2$).

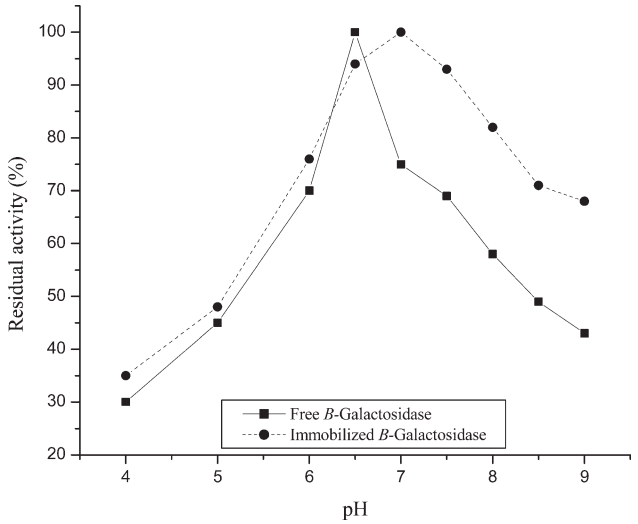


**Figure 5.** Effect of pH on $\beta$-galactosidase activity.

and nearly all of these groups were activated through the reaction with glyoxal cross-linker. Subsequently, the GP value was taken as a measurement for the degree of the fibers functionalization. The immobilization efficiency and retained activity of the immobilized $\beta$-galactosidase enzymes were investigated as a function of functionalization degree and the results were summarized in Table II. As we can see, the immobilization efficiency exhibited a significant increase from 53.9% to 98.5% by increasing the functionalization degree, which can be attributed to the high accessibility of the active aldehyde groups, which are able to covalently bond the $\beta$-galactosidase enzyme; this will of course reduce the amount of the enzyme lost during the washing step. On the other hand, increasing the degree of grafting and subsequently the degree of functionalization will obviously reduce the retained activity from 93.4% to 71.1%. This trend may be due to the multipoint attachment, which is a common observation in covalent immobilization techniques.[12] In this case, the covalent bonding of the enzyme protein from several points will of course slightly change the conformational structure of the protein and subsequently reduces the activity. Furthermore, increasing the amount of the immobilized enzyme may leads to overcrowding and protein–protein interactions, which cause a diffusional limitation of the substrate and products to and from the immobilized enzyme.

### Effect of pH on the *Activity*

The microenvironment of the enzyme could significantly influence the enzyme protein molecules. Because of the electronic parameters such as the surface charge of the support, the observed optimum pH for the immobilized enzyme may exhibit a difference from that of the free enzyme.[31] The retained activity of both free and immobilized $\beta$-galactosidase were studied against the pH in a range between 4 and 9 at 37○C and the results were presented in Figure 5. The obtained results showed that the optimum pH of the immobilized $\beta$-galactosidase by $S_2$ is 7 which is slightly higher than that of the free $\beta$-galactosidase, which presents the maximum ONPG hydrolytic activity at pH value of 6.5. This indicated that the $\beta$-galactosidase immobilization process on the modified silk fibers changes the optimum pH to more neutral value, which confirms that the distribution of hydrogen cations and hydroxide anions is not uniform in the direct vicinity to the immobilized enzyme and inside the bulk of the solution with higher negativity close to the immobilized $\beta$-galactosidase. Previous observations had been reported in the literatures.[32–34] In earlier study by Bayramoglu et al.,[35] in which $\beta$-galactosidase was immobilized on poly(glycidylmethacrylate–methylmethacrylate) magnetic beads, the pH shifted to neutral pH 7. In addition, Jochems et al.[6] prepared a mixed-matrix membrane containing zirconium dioxide for $\beta$-galactosidase immobilization by adsorption and the optimal pH was also changed from 6.5 to 7, which were in agreement with our current study.

**Table II.** Comparison of Immobilization Capacity and Activity of ***β***-Galactosidase onto Modified S-*g*-PAN Fibers

| Fibers | GP (%) | Immobilized enzyme (mg $g^{-1}$) | IE (%) | Enzyme activity (U $g^{-1}$) fiber | Activity retention (%) |
|---|---|---|---|---|---|
| $S_1$ | 35 | 45.3 | 53.9 | 8154.2 | 93.4 |
| $S_2$ | 87 | 75.5 | 90.2 | 12835.3 | 85.2 |
| $S_3$ | 130 | 82.5 | 98.5 | 11731.5 | 71.1 |

### Effect of *T*emperature

Temperature is one of the most vital parameters affecting the enzyme activity. In general, raising the temperature is usually accompanied by increasing the reaction rate. However, in the case of enzymatic reactions, the reaction exhibits an optimum temperature at which the enzyme shows its maximum activity, above this temperature the enzyme protein may undergo denaturation, which will subsequently causes significant conformational changes accompanied by a dramatic decrease in the activity.

The activity of both free and immobilized $\beta$-galactosidase was investigated as a function of temperature at pH 6.5 and the results were shown in Figure 6. As we can see, the optimal temperature of the immobilized $\beta$-galactosidase on $S_2$ was 40○C, which present a significant shift when compared to the free

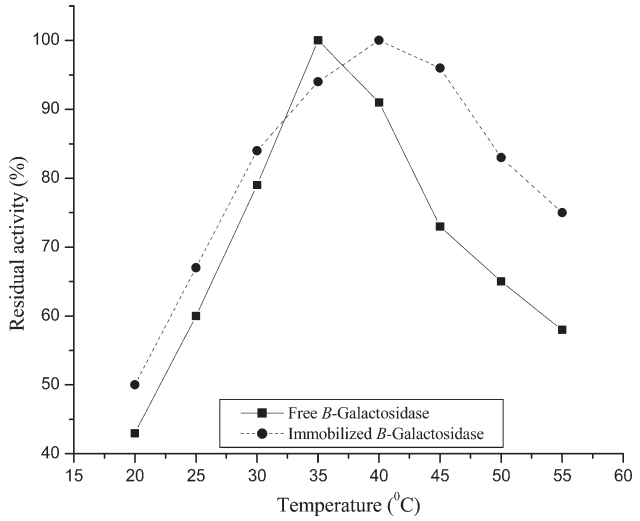


**Figure 6.** Effect of temperature on β-galactosidase activity.

enzyme, which showed its optimal activity at 35○C. In addition, the immobilized β-galactosidase keeps comparatively higher levels of the retained activity above 40○C. These results were in accordance with the observation recorded by Tanriseven and Dogan[37] where β-galactosidase was immobilized on alginate gelatin fibers and Husain et al.[1] in which β-galactosidase was immobilized on ZnO nanoparticles. The elevation of the thermal stability of β-galactosidase after immobilization could be explained as follows; the covalent immobilization of the β-galactosidase on the polymeric carrier fibers support will of course decreases the flexibility of the protein macromolecules toward conformational changes, which will increase the resistance to undergo denaturation.[36] Furthermore, the relatively high thermal stability of the immobilized enzyme decreases the limitation of substrate and product diffusion to and from the immobilized enzyme at relatively higher temperatures, which will obviously enhance the enzyme activity.

### Kinetic *P*roperties

Anticipation of the kinetic parameters regarding the enzymatic reactions is of great importance. For this reason, the initial reaction rate was studied against the substrate concentration. Here, in this study, a series of various ONPG substrate concentrations between 0.2 and 1.6 mmol $L^{-1}$ was utilized to evaluate the kinetic parameters by plotting the initial rate as a function of the substrate concentration [Figure 7(a)] followed by fitting the results with Michael's–Menten kinetics *eq.* (4).[12]

$$1/v_0 = K_m/v_{\max} .1/[S]+1/v_{\max} \quad (4)$$

where $v_0$ (U $mg^{-1}$) is the initial rate, [*S*] is the ONPG concentration (mmol $L^{-1}$), $K_m$ (mmol $L^{-1}$) is Michael's constant, and $v_{\max}$ (U $mg^{-1}$) is the maximum rate.

The kinetic parameters $K_m$ and $v_{\max}$ were then evaluated by plotting the reciprocal of the ONPG concentration against the reciprocal of the initial rate [Figure 7(b)] and results were summarized in Table III.

The observed increase in $K_m$ values with the accompanied lowering in $v_{\max}$ values after immobilization of the β-galactosidase

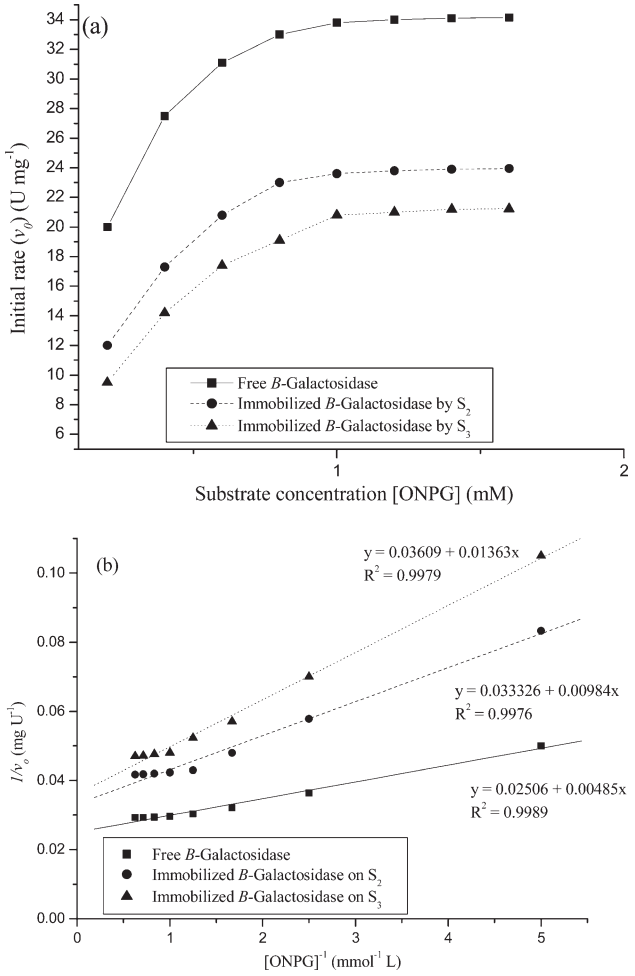


**Figure 7.** (a) Variation of initial hydrolysis rate of ONPG versus substrate concentration. (b) Lineweaver–Burk plot of free and immobilized β-galactosidase.

enzymes could be explained as a result of the limited accessibility of the substrate after the fixation on the polymeric carrier support. As the enzyme covalently bonded to the modified silk fibers, its freedom to bind with the substrate will be restricted, which results in a lowering in the activity appeared in the observed changes in both $K_m$ and $v_{\max}$. Similar observations were previously reported by Pahujani et al.[13] where lipase was immobilized on modified nylon-6 polymeric support, Yigitoglu et al.[14] where C.R. lipase were immobilized on grafted polyester

**Table III.** Kinetic Parameters for Both Free and Immobilized **β**-Galactosidase

| Enzyme form | $K_m$ (mmol $L^{-1}$) | $v_{\max}$ (U $mg^{-1}$) |
|---|---|---|
| Free β-galactosidase | 0.193 | 93.00 |
| Immobilized β-galactosidase by $S_2$ | 0.295 | 30.00 |
| Immobilized β-galactosidase by $S_3$ | 0.377 | 27.71 |

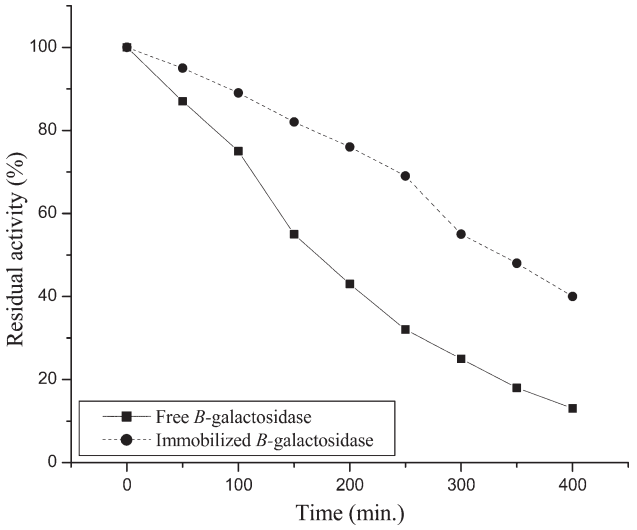


**Figure 8.** Thermal stability of free and immobilized β-galactosidase.

fibers and Ansari et al.[38] where β-galactosidase was immobilized on modified nano-Ag particles.

### Stability and Reusability

One of the important parameters when we evaluate the immobilization process is the storage stability. As mentioned before, the covalent binding of the enzymes onto polymeric supports may cause a restricted flexibility toward performing a dramatic variation in the conformational structure of the enzyme protein, which will in turn enhance the stability toward denaturation.[13] The storage stability of both free and immobilized β-galactosidase was measured at 50○C by testing the relative enzymatic activities for 6 h. Figure 8 summarizes the obtained results and as we can see β-galactosidase exhibited better performance throughout the 6 h by keeping about 40.5% of its original activity when compared to the free β-galactosidase, which losses 87% of its activity during the same period of study. The obtained results confirm that the immobilization of β-galactosidase onto the modified silk fibers obviously enhances the enzyme stability.

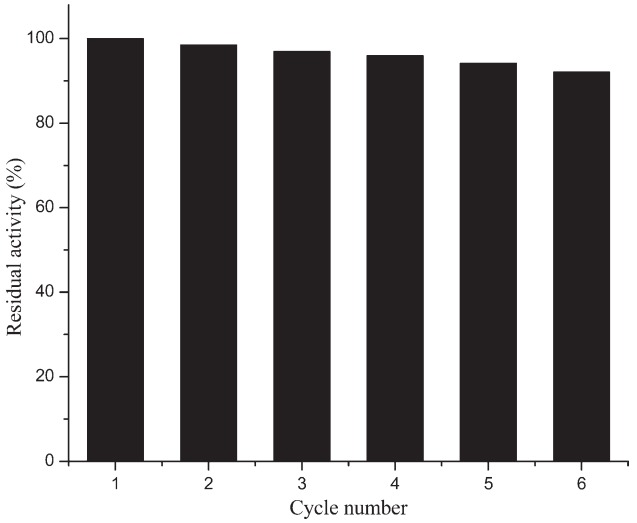


**Figure 9.** Operating stability of immobilized β-galactosidase.

The reusability of the immobilized enzyme is also one of the most significant parameters regarding the industrial applications. To anticipate the operational stability of the immobilized β-galactosidase on the $S_2$ fibers, a fresh sample was employed in the reaction and withdrawn upon the reaction completion, stored at 5○C for 24 h and reused again with new ONPG substrate sample. The process was repeated for six consecutive cycles and the residual activity was estimated at the end of each cycle. Figure 9 summarizes the reusability results and as can be seen the immobilized β-galactosidase retained about 92% of its activity after the sixth cycle which indicates that the immobilization process provides a good performance in the industrial applications.

## CONCLUSION

Here, in this study, polymeric support based on the natural protein silk fibers was prepared and characterized for covalent immobilization of β-galactosidase from *Escherichia coli*. The silk fibers were grafted using polyacrylonitrile in presence of benzophenone as a photo-initiator. The grafted fibers were then activated by treatment with hydrazine hydrate followed by glyoxal cross-linker. FTIR spectra, scanning electron microscope (SEM) in addition to the staining test derived from the Coomassie protein assay were utilized for investigation of the modification and immobilization steps. Also, the activity of both free and immobilized β-galactosidase was evaluated as a function of the various important parameters like grafting percentage, pH and temperature. The optimal pH was shifted to 7 after immobilization, which was slightly higher than that of the free enzyme (optimal pH at 6.5). Also the optimal temperature of the immobilized enzyme increased by 5○C compared to the free enzyme, which indicate the improvement of the thermal stability. In addition, the kinetic parameters $K_m$ and $v_{max}$ for both free and immobilized enzyme were anticipated using Michael's–Menten equation and the results indicates the increasing of $K_m$ and lowering of $v_{max}$ after the immobilization. The results in this study indicated that the prepared modified woven silk fibers could be used effectively as a polymeric support for immobilization of β-galactosidase.

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