Polyester fabric modification by chemical treatment to enhancing the β-glucosidase immobilization

Yaaser Q. Almulaikya,b,*

a University of Jeddah, College of Science and Arts at Khulais, Department of Chemistry, Jeddah, Saudi Arabia
b Chemistry Department, Taiz University, Taiz, Yemen

ARTICLE INFO
Keywords:
β-glucosidase
Chemical immobilization
Polyester fabric
Hydrazine hydrate

ABSTRACT
This work reports the first approach to immobilizing the β-glucosidase enzyme on a modified polyester fabric support matrix. Herein, polyester fabric was successfully fabricated with hydrazide groups incorporated with graphene oxide, followed by glyoxal as the crosslinker. Various techniques, including Fourier transform infrared spectroscopy, scanning electron microscopy, thermogravimetric analysis, differential scanning calorimeter, and zeta potential analysis, were used to investigate their microstructural, dispersive, thermal, and physicochemical properties. β-glucosidase immobilization exhibited maximum activity at pH 6.0 with an immobilization yield (89.5%), immobilization efficiency (92%), and enzyme activity yields (82.3%). After fifteen reaction cycles, the remaining enzyme activity was 59%. Stored at 4 °C, immobilized β-glucosidase retained 74% of its activity, compared to a retain of 43% for soluble β-glucosidase, during the 6-weeks period. Soluble and immobilized enzyme exhibited similar optimal catalytic temperature at 60 °C, while the optimal catalytic pH was 5 and 6, respectively. Both soluble and immobilized β-glucosidase presented Michaelis–Menten kinetics with Vmax values of 1.82 and 2.94 U/mg, and Km values of 2.94 and 5.15 mM, respectively. This research provided a potential directed immobilization method for β-glucosidase, and robust biocatalyst for industrial applications.

1. Introduction
Environment friendliness has heightened the demand for renewable resources to replace fossil-derived supplies. As the only viable renewable carbon source capable of transforming into fuels and value-added chemicals, biomass might be a potential alternative to depleting global fossil energy supplies [1, 2]. To meet environmental and long-term energy security demands, lignocellulosic biomass has been identified as a possible low-cost, renewable source for biofuel production [3]. It has the benefit of being the most plentiful and the cheapest biomass, and the only one that can grow on non-agricultural terrain without competing with food [4]. Cellulose is the most abundant lignocellulosic in plant biomass, and it provides the essential raw materials for bioethanol production [5]. Due to its ability to hydrolyze the end dimer of cellobiose, β-glucosidase is a crucial part of the cellulase multienzyme complex. Due to their outstanding selectivity and specificity as well as their high efficiency under mild conditions, enzymatic biocatalysts are receiving a growing interest [6, 7]. Enzymatic biocatalysts are an attractive method for dealing with cellulose conversion. But free enzymes are unstable and difficult to recover, resulting in high costs and low production efficiency [8, 9]. One of the main challenges that must be overcome in biocatalysts to reduce the high cost of enzymes is the usage of enzymes in soluble form, which has several drawbacks such as its limited stability and difficulties recovering the enzyme after utilizing it in repetitive cycles [10]. The immobilization of the enzyme on a solid support is one approach to overcome these restrictions; utilizing this technique, one may benefit from heterogeneous catalyzes, such as the ability to recycle the biocatalyst and improve the stability of the enzymes against temperature and pH fluctuations [11]. Some parameters that must be considered during immobilization include enzyme choices, support type, and immobilization parameters including temperature, ionic strength, pH, and duration. Immobilizing enzymes on textiles was found to be a promising way to improve their stability and reusability. Several enzymes have been effectively immobilized on modified textiles, retaining high enzyme activity, such as peroxidase immobilized on amidrazone modified acrylic fabric [12], α-amylase immobilized on amidrazone acrylic fabric [13], and laccase immobilized on chitosan/polyamide nanobbers [14]. Textile provides size, form, and orientation versatility, allowing the random creation of reactors appropriate for a given purpose. Additionally, fabric textiles...
make it possible to separate immobilized catalysts from the reaction medium in a complete and effective way. Furthermore, the textile surface’s open, active, and porous structure allows for minimal pressure drop, allowing for efficient reaction mixture diffusion, mass transfer, and efficient substrate turnover [15]. β-Glucosidase [EC 3.2.1.21] is one of the most important enzymes required for converting cellulose to glucose. The hydrolytic breakdown of the β-glycosidic bond between glucose and aryl or alkyl glycone residues is catalyzed by this enzyme, which belongs to the hydrolase family [16]. β-Glucosidase was immobilized using a variety of methods; for instance, it was immobilized on magnetic nanoparticles [17], on MnO2 magnetic nanomaterial [18], and in SiO2 nanoparticles using glutaraldehyde as a crosslink [19]. To the best of our knowledge, the immobilization of β-glucosidase on polyester treated with hydrazide hydrate and graphene oxide has not been reported to date. Thus, in this study, β-Glucosidase was immobilized on modified polyester for improving and enhancing reusability and stability. The morphological structure of obtained support was investigated using SEM, FTIR, TGA, DSC, and zeta potential analysis before and after immobilization. The biochemical properties of soluble and immobilized enzyme were also studied.

2. Experimental

2.1. Materials

β-glucosidase from almonds, 4-nitrophenyl-β-D-glucopyranoside (p-NPG), p-nitrophenol, hydrazide hydrate 80%, graphene oxide (GO), Poly(vinyl alcohol) (PVA) MW 96000, 99% hydrolyzed, and glyoxal were supplied from Sigma Aldrich. Polyester woven fabric (PET) (195 g/m2) was supplied by Misr El-Mahalla Co., Egypt. Other chemicals used in this study were supplied from Merck Co. (Germany).

2.2. Polyester modification

The treatment of a particular weight of polyester (PET) using 12% hydrazide hydrate in methanol at a liquor-to-goods ratio of 30:1 was performed at 65 °C for 40 min. The treated sample was carefully rinsed in water and air-dried. The treated polyester (PET-hydrazide) was immersed overnight in 50 mL chloroform containing 2 mg/ml graphene oxide. The graphene-coated treated polyester (PET-hydrazide/GO) was dried at 60 °C for 1 h, then sonicated in PVA (10 mg/ml) at 60 °C for 1 h. The obtained system was stirred in glyoxal (2% v/v) for 4 h at 40 °C, then rinsed with water and air-dried.

2.3. Immobilization process

The immobilization was carried out using the approach described earlier [20]. In Na-acetate buffer (50 mM), pH 6.0 or Tris–HCl, pH 7.0 or 8.0, immobilization was achieved by mixing enzyme end-over-end at 100 rpm with 1 g of treated polyester (PET-hydrazide/GO). For 12 h, the immobilization procedure took place at room temperature. The immobilized enzyme on PET-hydrazide/GO was withdrawn and dried at room temperature. The amount of loaded protein was calculated by subtracting the quantities of protein in the aqueous solution following the immobilization procedure from the amount of protein introduced. The following equations were used to estimate the β-glucosidase activity (unit/g support) and immobilization yield (%). The protein concentration was determined according to Bradford method using bovine serum albumin as standard [21].

\[
\text{Immobilization Yield (IY\%)} = \frac{A1 - A2}{A1} \times 100
\]

where A1 is the amount of protein introduced and A2 is protein in the supernatant after immobilization.

2.4. β-Glucosidase activity assay

According to the method of Narasimha, the activity of β-glucosidase was determined by estimating the liberation of p-nitrophenol using the substrate p-NPG [22]. Briefly, 0.1 mL of soluble β-glucosidase enzyme or 10 mg of immobilized β-glucosidase was added to 0.9 mL of 5 mM p-NPG (prepared in 100 mM sodium acetate buffer, pH 6.0). After incubating the reaction mixture for 5 min at 37 °C, 0.5 mL of 1M Na2CO3 solution was added to terminate the reaction and recorded the absorbance at 405 nm. The quantity of enzyme generating 1 μmol of p-nitrophenol per min was defined as one unit of β-glucosidase activity.

2.5. Morphological characterization

Field emission scanning electron microscopy (FESEM, JEOL JSM 7600F FEG-SEM) was used to examine the morphological characteristics of the PET, PET-hydrazide/GO and PET-hydrazide/GO after immobilization. All samples were gold-coated, held in a holder with Quanta stubs, and analyzed under a vacuum. The chemical composition of all samples was investigated using Fourier-transform infrared spectroscopy (ATR–FTIR, PerkinElmer Spectrum 100). Using the Shimadzu DTA/TGA-50, Japan, with a heating rate of 10 °C/min under nitrogen, thermogravimetric-derivative thermogravimetric and differential scanning calorimetry analysis (TGA-DTG and DSC) of the samples was conducted. The zeta potential of the samples was determined using dynamic light scattering (Entgris, Nicomp Nano Z3000, Billerica, MA, USA).

2.6. Biochemical and physicochemical characterization

2.6.1. Influence of temperature and pH

To determine the optimum temperature, the activity of the soluble and immobilized enzyme was measured over a wide range of temperatures (30–80 °C), as described in the test protocol. To determine the optimum pH, the activity of the soluble and immobilized enzyme was measured in 100 mM of Na-acetate buffer (pH 4.0–6.0), phosphate buffer (pH 6.5–8.0), and Tris-HCl buffer (pH 8.5–9.0). The initial activity was compared to the relative activity on the assumption that the initial activity was 100%.

2.6.2. Determination of kinetic parameters

Lineweaver-Burk plots were used to estimate the kinetic parameters (Km and Vmax) of the soluble and immobilized enzyme. Plotting different p-NPG concentrations (4–10 mM) was performed for this investigation. In analyzing enzyme kinetics data, Km and Vmax play a very important role. Linearization of the kinetic equations is still a common practice for determining these parameters. Km and Vmax were determined via plotting 1/V against I/[S] giving a straight line, y = mx + b; b = 1/Vmax and m = Km/Vmax.

2.6.3. Reusability and storage stability

One of the most significant benefits of the immobilization procedure is that the immobilized enzyme can be recycled. Briefly, 10 mg of immobilized enzyme was mixed with the p-NPG solution and measured as described in the test protocol. At the end of the reaction, the immobilized enzyme was isolated and washed with distilled water before being blended with a new substrate solution. This method was carried out 15 times to assess the reusability of the immobilized enzyme. The initial activity was compared to the relative activity on the assumption that the initial activity was 100%.
To assess storage stability, the soluble and immobilized enzyme was kept at 4 °C in 100 mM Na-acetate buffer (pH 6), and activity was measured every 1-week intervals for 6 weeks. The initial activity was compared to the relative activity on the assumption that the initial activity was 100%.

3. Results and discussion

It was predicted that hydrazine hydrate activation of polyester fabric (PET) would produce active hydrazide sites accessible for enzyme immobilization. Due to the superior mechanical properties and surface areas of graphene oxide (GO), the treated polyester (PET-hydrazide) was coated with GO and stabilized with PVA. PVA serves as a reducing agent, growth regulator, nanoparticle dispersant, and surface stabilizer [23]. The Schiff’s base crosslinked glyoxal was synthesized to react with an amino group of enzymes (Figure 1). The immobilization process was performed at different pH media, as shown in Table 1, it should be noted that in this work the immobilized enzyme exhibited maximum activity at pH 6.0 and gives rise to higher enzyme activity yields (82.3%), immobilization yields (89.5%), and immobilization efficiency (92%). Zheng et al. [24] reported that the magnetic chitosan microspheres was used as support for β-glucosidase. The optimal pH for immobilization was at pH 4.8 with an immobilization efficiency of 90.2% and enzyme activity yield of 83.2%. This immobilization yield has been reported also by

![Figure 1. Schematic representation for the synthesis of PET-hydrazide/GO and enzyme immobilization.](image)
β-glucosidase from *A. niger* when adsorbed on MANAE-agarose [25]. This immobilization efficiency at pH 6 is related to the ionizable groups of the enzyme which has more cationic groups at pH 6 than at pH 7 because its isoelectric point is 8.7 [26]. Changes in the ionic character of the enzyme surface can impact its interaction with the support surface, altering adsorption properties, orientation, or localized ionic repulsion/attraction [27].

The FTIR of the pristine PET and the treated samples was shown in Figure 2. The PET exhibits distinctive peaks at 1709 cm\(^{-1}\) for carbonyl ester, at 2967, 2894 cm\(^{-1}\) for C–H stretching, at 1244, 1089 cm\(^{-1}\) for C–O ester group vibration, and at 722 cm\(^{-1}\) for C–H aromatic out-of-plane bending vibration. Similar FTIR results have been reported for polyester [28, 29, 30]. After being treated with hydrazine/GO treatment, these peaks either broadened with the emergence of new peaks due to N–H amine at 3206, 3431 cm\(^{-1}\), and shoulder at neighboring 1704 cm\(^{-1}\) due to carbonyl amide or shifted with increased intensity due to band overlap for those attributable to ester, amide, and amino groups, as shown in Figure 1. This indicates the success of hydrazide formation on the surface of polyester (PET-hydrazide). The O–H bond tension vibrations were represented by the band at 3363 cm\(^{-1}\), which is supplemented by the C–OH band at 1244 and 1089 cm\(^{-1}\). This is due to the hydroxyl groups of the GO that overlay with that of C–O vibrations of the ester group for PET. After enzyme immobilization, the spectrum is featured for the band peak at 1653 cm\(^{-1}\) to C–O stretch (amide I). The band peaks have appeared at 2931 cm\(^{-1}\) refers to C–H aliphatic stretching, and at 1513 cm\(^{-1}\) refers to N–H in-plane bending and C–N stretch, amide II). Amide III spectrum for enzyme has appeared band at 1319 cm\(^{-1}\) (C–H and N–H deformation vibrations).

The zeta potentials of the PET, PET-hydrazide/GO, and PET-hydrazide/GO/β-glucosidase were analyzed by dynamic light scattering. Figure 3 illustrates the zeta potentials of all tested samples at pH 7.0. The zeta potential value for PET was found to be −10.8 mV. After PET modification, the zeta potential dropped to −17.7 mV. This occurred as a result of the active –OH groups introduced by the hydrazide/GO modification to the surface of PET. Finally, the zeta potential of the PET-hydrazide/GO/β-glucosidase was measured to be −15.6 mV. The isoelectric point of β-glucosidase is known to be pH 8.7 [26], therefore, the structure of the enzyme exhibits a net positive charge at pH values below 8.7. As a result, depending on the charge of β-glucosidase, the total surface charge

### Table 1. Immobilization yields (IY), activity yields (AY), and immobilization efficiency (IE) of β-glucosidase loaded on modified polyester.

| pH | Protein introduced (mg) | Immobilized protein mg/g support | IY (%) | Initial Activity (Units) | Immobilized Enzyme Activity (Units) | AY (%) | IE (%) |
|----|-------------------------|---------------------------------|--------|--------------------------|-------------------------------------|--------|--------|
| 6  | 13.4 ± 0.95             | 11.99 ± 0.42                    | 89.5 ± 1.52 | 201 ± 2.3                | 105.4 ± 1.49                        | 82.3 ± 0.87 | 92 ± 1.46 |
| 7  | 8.51 ± 0.35             | 63.5 ± 1.32                     | 78.1 ± 1.95 | 108.9 ± 2.1              | 60.5 ± 1.69                         | 51.8 ± 0.94 | 63 ± 1.06 |
| 8  | 6.8 ± 0.21              | 50.7 ± 1.43                     | 76.2 ± 2.15 | 152 ± 2.3                | 21.13 ± 1.05                        | 26.8 ± 0.94 | 37 ± 0.93 |

---

**Figure 2.** ATR-FTIR spectra of PET, PET-hydrazide/GO, and PET-hydrazide/GO/Enzyme.

**Figure 3.** Zeta potential value for PET, PET-hydrazide/GO, and PET-hydrazide/GO/Enzyme.
increased following β-glucosidase immobilization. The values of the zeta potential were shifted when the functional groups and surface changes change. The results show that surface modification and β-glucosidase immobilization were accomplished successfully.

The morphological surface structure of the pristine PET, the effects of the hydrazine hydrate/GO treatment, and the morphological change following enzyme immobilization are shown in Figure 4. Figure 4a exhibits the PET’s morphology with a uniform and smooth surface. Following treatment with hydrazine hydrate/GO, a significant alteration can be seen on the surface of PET, as depicted in Figure 4b. The PET-hydrazide/GO surface structure was revealed to be uneven and rough. As demonstrated in Figure 4c for sample PET-hydrazide/GO/enzyme, further alterations, such as minor fractures, could still be seen in the treated polyester fabric following enzyme immobilization. On our previous studies, we emphasized the change in the surface of acrylic textiles following chemical treatment and immobilization as a strategy for the efficacy of the enzyme immobilization process [12, 13, 31].

The TGA-DTG profiles of PET, PET-hydrazide/GO, and PET-hydrazide/GO/enzyme are shown in Figure 5(a, b). The onset degradation (T<sub>Onset</sub>), temperature at 50% mass loss (T<sub>50</sub>), the thermal decomposition temperature (T<sub>End</sub>) and the Inflection point of all samples tabulated in Table 2. The onset degradation temperature (T<sub>Onset</sub>) for PET-hydrazide/GO/enzyme shows an increase of about 3°C as compared to PET and PET-hydrazide/GO. The immobilization enzyme decreased the thermal decomposition temperature (T<sub>End</sub>). As can see in Table 2, the significant change rate on the curve is known as the inflection point at 441.2°C, 442.8°C, and 439.7°C for PET, PET-hydrazide/GO, and PET-hydrazide/GO/enzyme and the total mass loss of 69%, 62.5%, and

| Sample               | DSC       | TGA-DTG               |
|----------------------|-----------|-----------------------|
|                      | Degradation process |                   |
|                      | T<sub>Onset</sub> °C | T<sub>Peak</sub> °C | T<sub>End</sub> °C | ΔH J/g | T<sub>Onset</sub> °C | T<sub>50</sub> °C | T<sub>End</sub> °C | Inflection point, °C | Mass Change % |
| PET                  | 489       | 606                   | 630               | 182    | 433.3          | 445.8             | 453.2             | 441.2               | −79.55        |
| PET-hydrazide/GO     | 540       | 627                   | 645               | 72.7   | 433.0          | 442.9             | 452.9             | 442.8               | −80.95        |
| PET-hydrazide/GO/Enzyme | 594     | 621                   | 633               | 13.6   | 436.0          | 442.3             | 444.5             | 439.7               | −82.10        |

Figure 4. Low and high magnification FESEM images of a) PET, b) PET-hydrazide/GO, c) PET-hydrazide/GO/Enzyme.

Figure 5. a) TGA and b) DTG thermograms of PET, PET-hydrazide/GO, and PET-hydrazide/GO/Enzyme.

Table 2. Thermogravimetric degradation behavior of PET, PET-hydrazide/GO, and PET-hydrazide/GO/Enzyme.
67.6%, respectively. This significant variation in the thermal behavior of the support under study is an indication of the success of PET treatment and enzyme immobilization. This result is in accordance with studies reported for polyester/CuO nanocomposites [32] and polyester/silica [33]. Figure 6 shows the DSC curve of PET, PET-hydrazide/GO, and PET-hydrazine/GO/enzyme. It displays the thermograph and enthalpy change in relation to temperature. The DSC thermogram of PET, PET-hydrazide/GO, and PET-hydrazine/GO/enzyme displayed endothermic peaks (T_peak) at 606, 627, and 621 °C, respectively, and the enthalpy of fusion (Δ_H) was 182, 72.7, and 13.6 J/g, respectively. Table 2 illustrates the onset and end temperatures of the latent heat of fusion for all samples. The thermogram of β-glucosidase exhibited a broad, endothermal band at 450–740 °C, which might be due to the protein’s random chains and α-helices opening, causing denaturation at such high temperatures [34]. This thermal stability in the PET-hydrazide/GO/enzyme sample could be attributed to the enzyme’s strong electrostatic association with the functionalized PET. The high affinity between the enzyme’s amino groups and the glyoxal’s carbonyl groups resulted in enhanced heat stability of the polyester network support. This also validates the immobilization of enzyme on the PET-hydrazide/GO surface.

Many immobilized enzyme applications require operating under temperature and pH conditions that are far from optimal. Figure 7a shows the results of assessing the activity of soluble and immobilized β-glucosidase enzyme at various pH levels. As shown, the soluble β-glucosidase exhibited the highest activity at pH 5.0, whereas the immobilized β-glucosidase had the optimal activity at pH 6.0. This pH profile shift can be explained by the electrostatic interactions between the cationic-modified polyester support and the microenvironment of the cross-linked β-glucosidase, which will lead to unequal concentrations of −OH/−H ions around the fixed β-glucosidase molecules and within the working solution [35]. Moreover, the immobilized β-glucosidase demonstrated a broader pH profile, which could be attributed to the protection given by the modified polyester support, as well as product buildup, which will, of course, impact the pH level in the vicinity of the immobilized β-glucosidase [35]. Similar pH profiles were observed in previous studies by Naseer et al [36] where β-glucosidase was cross-linked immobilized on SiO2 nanoparticles. Gupta et al [37] reported that the alginate was employed as solid support for the encapsulation of β-glucosidase, and Çelik et al [38] reported that the enzyme was effectively immobilized on chitosan-multiwalled carbon nanotubes. Additionally, a series of working solutions with temperature changes between 20 and 80 °C were used to evaluate the activity of immobilized and soluble β-glucosidase. The activity profile is shown in Figure 7b. The optimal temperature for both immobilized and soluble β-glucosidase was 60 °C. However, at temperatures over 60 °C, the immobilized β-glucosidase exhibited a comparatively greater residual activity compared to the soluble β-glucosidase, indicating that the β-glucosidase enzyme has higher thermal stability after being covalently immobilized onto the modified PET. This is exemplified by the robust binding between the β-glucosidase molecules and the modified PET, which limits the conformational changes that might occur within the β-glucosidase protein macromolecules when exposed to high temperatures and hence prevents denatured active catalytic sites [39]. This result agrees with previous reports. For example, Gupta et al [37], Verma et al [40] and Patel et al. [41] observed that 60 °C was the optimal temperature for both the soluble and immobilized β-glucosidase.

Using different concentrations (4–10 mM) of pNPG as the substrate, the influence of substrate concentration on the rate catalyzed by soluble and immobilized β-glucosidase was investigated (Figure 8). The Lineweaver-Burk plot was used to determine the Michaelis constant (Km) and the maximal reaction velocity (Vmax) of soluble and immobilized enzyme. The observed Km value of the immobilized β-glucosidase was 5.15 mM, greater than that of the soluble β-glucosidase (2.94 mM). Similar results for immobilized β-glucosidase on chitosan-multiwalled carbon nanotubes were reported by Çelik et al [38]. While Gupta et al [37] reported that the Km and Vmax encapsulated β-glucosidase was 5.0 mM and 0.64 U/mg. After immobilization, Km rises, indicating that the

---

**Table 2** illustrates the onset and end temperatures of the latent heat of fusion for all samples. The thermogram of β-glucosidase exhibited a broad, endothermal band at 450–740 °C, which might be due to the protein’s random chains and α-helices opening, causing denaturation at such high temperatures [34]. This thermal stability in the PET-hydrazide/GO/enzyme sample could be attributed to the enzyme’s strong electrostatic association with the functionalized PET. The high affinity between the enzyme’s amino groups and the glyoxal’s carbonyl groups resulted in enhanced heat stability of the polyester network support. This also validates the immobilization of enzyme on the PET-hydrazide/GO surface.

Many immobilized enzyme applications require operating under temperature and pH conditions that are far from optimal. Figure 7a shows the results of assessing the activity of soluble and immobilized β-glucosidase enzyme at various pH levels. As shown, the soluble β-glucosidase exhibited the highest activity at pH 5.0, whereas the immobilized β-glucosidase had the optimal activity at pH 6.0. This pH profile shift can be explained by the electrostatic interactions between the cationic-modified polyester support and the microenvironment of the cross-linked β-glucosidase, which will lead to unequal concentrations of −OH/−H ions around the fixed β-glucosidase molecules and within the working solution [35]. Moreover, the immobilized β-glucosidase demonstrated a broader pH profile, which could be attributed to the protection given by the modified polyester support, as well as product buildup, which will, of course, impact the pH level in the vicinity of the immobilized β-glucosidase [35]. Similar pH profiles were observed in previous studies by Naseer et al [36] where β-glucosidase was cross-linked immobilized on SiO2 nanoparticles. Gupta et al [37] reported that the alginate was employed as solid support for the encapsulation of β-glucosidase, and Çelik et al [38] reported that the enzyme was effectively immobilized on chitosan-multiwalled carbon nanotubes. Additionally, a series of working solutions with temperature changes between 20 and 80 °C were used to evaluate the activity of immobilized and soluble β-glucosidase. The activity profile is shown in Figure 7b. The optimal temperature for both immobilized and soluble β-glucosidase was 60 °C. However, at temperatures over 60 °C, the immobilized β-glucosidase exhibited a comparatively greater residual activity compared to the soluble β-glucosidase, indicating that the β-glucosidase enzyme has higher thermal stability after being covalently immobilized onto the modified PET. This is exemplified by the robust binding between the β-glucosidase molecules and the modified PET, which limits the conformational changes that might occur within the β-glucosidase protein macromolecules when exposed to high temperatures and hence prevents denatured active catalytic sites [39]. This result agrees with previous reports. For example, Gupta et al [37], Verma et al [40] and Patel et al. [41] observed that 60 °C was the optimal temperature for both the soluble and immobilized β-glucosidase.

Using different concentrations (4–10 mM) of pNPG as the substrate, the influence of substrate concentration on the rate catalyzed by soluble and immobilized β-glucosidase was investigated (Figure 8). The Lineweaver-Burk plot was used to determine the Michaelis constant (Km) and the maximal reaction velocity (Vmax) of soluble and immobilized enzyme. The observed Km value of the immobilized β-glucosidase was 5.15 mM, greater than that of the soluble β-glucosidase (2.94 mM). Similar results for immobilized β-glucosidase on chitosan-multiwalled carbon nanotubes were reported by Çelik et al [38]. While Gupta et al [37] reported that the Km and Vmax encapsulated β-glucosidase was 5.0 mM and 0.64 U/mg. After immobilization, Km rises, indicating that the

---

**Figure 6.** DSC scans of PET, PET-hydrazide/GO, and PET-hydrazide/GO/Enzyme.

**Figure 7.** a) effect of pH, and b) effect of temperature on the soluble and immobilized enzyme. Each point represents the average of three experiments.
immobilized enzyme has a perceptibly lower affinity for its substrate than the soluble enzyme. This might be due to active site support steric hindrance, a lack of enzyme flexibility required for substrate binding, or diffusional resistance to substrate transport [42, 43]. Furthermore, an increased Vmax was observed for immobilized β-glucosidase (2.94 U/mg) compared to soluble form (1.82 U/mg). This could be due to when the substrate concentration in the bulk solution increasing, the rate of substrate diffusion to the enzyme may grow to a limiting value. If an immobilized enzyme achieves this limiting value after being totally substrate-saturated, then its apparent Vmax value will be higher than that in the free solution [44]. Similar results for immobilized β-glucosidase on polydimethylsiloxane were reported by Hernández-Maya et al [45].

El-Shishtawy et al [46] reported that the Vmax value of immobilized catalase on chitosan/ZnO was higher than that of soluble form.

Reusability is the most important quality of immobilized enzymes for both commercial and industrial applications. The cost of the enzyme can be reduced by immobilizing β-glucosidase to facilitate the ability to recycle it in a continuous batch-wise operation. As shown in Figure 9a, about 82% of residual activity was observed on the immobilized β-glucosidase after recycling for ten cycles. After testing the immobilized preparation fifteen times, the lowest activity, 59%, was observed. Enzyme denaturation is thought to be the cause of activity reduction [31]. To investigate storage stability, the soluble and immobilized enzyme was stored in 0.1M sodium acetate buffer, pH 6 at 4°C (Figure 9b). The shelf-life of the soluble and immobilized enzyme was evaluated by measuring activity over a 6-week period. During the six weeks of storage, the soluble β-glucosidase lost 57% of its activity, compared to a loss of 26% for the immobilized β-glucosidase. The immobilized enzyme’s higher stability possibly be attributable to the enzyme’s multipoint attachments to the solid support, which prevent time-dependent denaturation [47, 48]. Comparative results with other study reports are provided in Table 3.

### 4. Conclusions

In conclusion, this work developed an in-situ hydrazide/graphene oxide plating method for enzyme immobilization on polyester textiles that is simple, moderate, and highly effective. Its morphology, thermal, structure, and electrokinetic potential properties were determined. The immobilized enzyme had highly immobilization yield, immobilization efficiency, and enzyme activity yields. The results demonstrated that the immobilization technique improved pH tolerance with a shift in optimal pH (from 5.0 to 6.0), optimal temperature (60°C), storage stability (74% activity after 6 weeks of storage), and more significantly, reusability (59% activity after 15 cycles of reuse). These recently discovered characteristics of the immobilized β-glucosidase imply that immobilization by covalent bonding can improve the biotechnological potential of this biocatalyst for use in a wide range of industrial applications.
Y.Q. Almulaiky

Declarations

Author contribution statement

Yasera Q. Almulaiky: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by the University of Jeddah, Saudi Arabia, under grant No. (UJ-21-DR-50). The authors, therefore, acknowledge with thanks the university technical and support.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] A.D. King, D.J. Karoly, Climate extremes in Europe at 1.5 and 2 degrees of global warming, Environ. Res. Lett. 12 (2017), 114031.
[2] V. Menon, M. Rao, Trends in biocconversion of lignocellulosic: biofuels, platform chemicals & bioenergy concept, Prog. Energy Combust. Sci. 38 (2012) 522–550.
[3] Y. Zhu, G. Xu, W. Song, Y. Zhao, Z. Miao, R. Yao, J. Gao, Catalytic microwave pyrolysis of orange peel: effects of acid and base catalyzed products mixture on distribution, J. Energy Inst. 98 (2021) 172–178.
[4] B. Mazzoli, Metabolic engineering strategies for consolidated production of lactic acid from lignocellulosic biomass, Biotechnol. Appl. Biochem. 67 (2020) 61–72.
[5] R. Tiwari, L. Nain, N.E. Labrou, P. Shaula, Bioprospecting of functional cellulases from metagenome for second generation biofuel production: a review, Crit. Rev. Microbiol. 44 (2018) 244–257.
[6] K.M. Koeller, C.H. Wong, Enzymes for chemical synthesis, Nature 409 (2001) 232–240.
[7] R.A. Sheldon, J.M. Woodley, Role of biocatalysis in sustainable chemistry, Chem. Eng. J. 118 (2006) 801–807.
[8] M.M. Ureta, G.N. Martins, O. Figueira, P.P. Pires, P.C. Castillo, A. Gómez-Zavaglia, Recent advances in β-galactosidase and fructosyltransferase immobilization technology, Crit. Rev. Food Sci. Nutr. 61 (2021) 2659–2690.
[9] V. Veneta, F. Sanna, A. Costantini, R. Silvestri, S. Cimino, V. Califano, Mesoporous silica nanoparticles for β-glucosidase immobilization by templating with a green material: tannic acid, Microporous Mesoporous Mater. 302 (2020), 112003.
[10] N.R. Mohamed, N.H.C. Marraki, N.A. Buang, F. Huyop, R.A. Wahab, An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes, Biotechnol. Biotechnol. Equip. 29 (2015) 205–220.
[11] B.C. Rodrigues, D. Carballes, R. Morellon-stirling, R. Fernandez-lafuente, Stabilization of enzymes via immobilization: multipoint covalent attachment and other stabilization strategies, Biotechnol. Adv. 52 (2021), 107821.
[12] Y.Q. Almulaiky, R.M. El-Shishtawy, M. Aldhahri, S.A. Mohamed, M. Affi, W.H. Abdulaziz, J.A. Mahiyoub, Amidesamine modified acrylic fabric activated with cyanuric chloride: a novel and efficient support for horseradish peroxidase immobilization and phenol removal, Int. J. Biol. Macromol. 140 (2019) 949–958.
[13] A.R. Al-Najada, Y.Q. Almulaiky, M. Aldhahri, R.M. El-Shishtawy, S.A. Mohamed, M. Baeshen, S.A. Al-Harbi, Immobilization of α-amylase on activated amidosamine acrylic fabric: a new approach for the enhancement of enzyme stability and reusability, Sci. Rep. 9 (2019) 1–9.
[14] M. Maryskova, I. Ardao, C.A. García-González, L. Martínová, J. Rotkova, A. Sevcu, Polymerized chitosan nanofibers as support for the immobilization of Trametes versicolor laccase for the elimination of endocrine disrupting chemicals, Enzym. Microb. Technol. 89 (2016) 31–38.
[15] M.N. Morshed, N. Behary, N. Bouaziz, J. Guan, V.A. Nierstrasz, An overview on biocatalysts immobilization on textiles: preparation, progress and application in wastewater treatment, Chemosphere 279 (2021), 130481.
[16] J.M. Gómez, M.D. Romero, T.M. Fernández, E. Díez, Immobilization of β-glucosidase in fixed bed reactor and evaluation of the enzymatic activity, Bioproc. Biosyst. Eng. 35 (2012) 1399–1405.
[45] F.M. Hernández-Mayo, M.P. Canizares-Macas, Evaluation of the activity of β-glucosidase immobilized on polydimethylsiloxane (PDMS) with a microfluidic flow injection analyzer with embedded optical fibers, Talanta 185 (2018) 53–60.

[46] R.M. El-Shishtawy, N.S. Ahmed, Y.Q. Almulaiky, Immobilization of catalase on chitosan/ZnO and chitosan/ZnO/Fe2O3 nanocomposites: a comparative study, Catalysts 11 (2021) 825.

[47] G. Bayramoglu, A.U. Metin, B. Altuntas, M.Y. Arica, Reversible immobilization of glucose oxidase on polyaniline grafted polyacrylonitrile conductive composite membrane, Bioresour. Technol. 101 (18) (2010) 6881–6887.

[48] Y.Q. Almulaiky, N.M. Khalil, R.M. El-Shishtawy, T. Altalhi, Y. Algamal, M. Aldhahri, M.M. Mohammed, Hydroxyapatite-decorated ZrO2 for α-amylase immobilization: toward the enhancement of enzyme stability and reusability, Int. J. Biol. Macromol. 167 (2021) 299–308.