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Activity and storage stability of immobilized glucose oxidase onto magnesium silicate

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Abstract

Glucose oxidase (GOD) was covalently immobilized onto florisil (magnesium silicate) carrier via glutaraldehyde. Immobilization conditions were optimized: the amount of initial GOD per grams of carrier as 5 mg, pH as 5.5, immobilization time as 120 min and temperature as 10 °C. Under the optimized reaction conditions activities of free and immobilized GOD were measured. Free and immobilized GOD samples were characterized with their kinetic parameters, and thermal and storage stabilities. K_M and V_{max} values were 68.2 mM and 435 U mg GOD^{-1} for free and 259 mM and 217 U mg GOD^{-1} for immobilized enzymes, respectively. Operational stability of the immobilized enzyme was also determined by using a stirred batch type column reactor. Immobilized GOD was retained 40% of its initial activity after 50 reuses. Storage stabilities of the immobilized GOD samples stored in the mediums with different relative humidity in the range of 0–100% were investigated during 2 months. The highest storage stability was determined for the samples stored in the medium of 60% relative humidity. Increased relative humidity from 0% to 60% caused increased storage stability of immobilized GODs, however, further increase in relative humidity from 80% to 100% caused a significant decrease in storage stability of samples.

Keywords: Glucose oxidase; Magnesium silicate; Glutaraldehyde; Covalent immobilization; Relative humidity

1. Introduction

Glucose oxidase (EC 1.1.3.4) catalyses the oxidation of β-D-glucose by oxygen to D-glucono-1,5-lactone and hydrogen peroxide. It is a dimeric protein with a molecular weight of 160 kDa, containing one tightly but noncovalently bound flavin adenine dinucleotide (FAD) per monomer as cofactor and glycosylated with a carbohydrate content of 16% (w/w). GOD has a number of industrial applications due to its ability to remove glucose and O_2 or production of gluconic acid and H_2O_2. GOD is used for desugaring of eggs in egg-solids, production of reduced alcohol wines [1], removal of oxygen and browning control to increase storage time of fruit juices, purees [2], mayonnaise [3] and other tinned foods. GOD use is also an alternative way to production of H_2O_2 for textile bleaching [4] or milk pasteurization. Gluconic acid and its derivatives have been produced by using GOD in a large quantities for pharmaceutical and industrial applications [5]. Immobilization of GOD can offer several advantages including its reuse, ease in application of both batch and continuous systems, possibility of better control of reactions, ease in removal from the reaction medium and improved stability. GOD has been immobilized on numerous carriers by using different methods [6–22].

In the present study, GOD was immobilized covalently via glutaraldehyde on to magnesium silicate (florisil) which contains 15% MgO and 85% SiO_2. Florisil was chosen because of its basic property which may play a special role in neutralization of gluconic acid produced by GOD in the pores. Thus, preventing the dramatic decrease in pH of microenvironment may hinder immobilized GOD inactivation and enhance the stability of immobilized GOD. Florisil carrier was also chosen due to its being commercially available, inexpensive and stable support. Immobilization conditions

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including pH, temperature, initial GOD concentration and immobilization time were optimized. The properties including optimum pH, buffer concentration, temperature, thermal and storage stability of immobilized GOD were compared with that of free enzyme. To our knowledge, storage stability of immobilized GOD in different relative humidity medium has not been reported, so it was also investigated.

2. Materials and methods

GOD (Sigma Cat. No: G 0543, 24 mg GOD ml^{-1} and 350 U mg $protein^{-1}$), 3-aminopropiltrietoxysilane (APTES), Grade I aqueous glutaraldehyde solution (50%), glucose, dinitrosalicylic acid (DNSA) obtained from Sigma (St. Louis, MO), florisil (60–100 mesh, specific surface area 170–300 m^2 g^{-1}) obtained from Merck AG (Darmstadt, Germany) and all other chemicals used are analytical grade.

2.1. Activation of florisil carrier

Florisil as carrier was silanized with APTES and activated via glutaraldehyde [23] before GOD immobilization. Florisil was washed with 5% (v/v) HNO_3 solution at 80–90 °C for 60 min followed by rinsing with distilled water and drying overnight at 120 °C. To 1 g of the carrier 25 ml of 4% solution of APTES in acetone (v/v) was added and evaporated to dryness at 45 °C for 24 h and then heated to 115 °C overnight. 25 ml of 2.5% (v/v) glutaraldehyde solution prepared with potassium phosphate buffer (50 mM, pH 7.0) was added onto 1 g carrier separately and the reaction was allowed to continue for 2 h. The colour of the carrier changed to magenta or tan and it was washed exhaustively with distilled water on a Buchnel funnel and dried.

2.2. Optimization of immobilization conditions

2.2.1. Immobilization pH

To investigate optimum immobilization pH, onto 0.2 g of activated carrier was added 2 ml of 0.1 mg ml^{-1} GOD prepared in 50 mM buffer solutions at different pH values (3.5–8.0). Immobilization mixture was kept at 20 °C for an hour and shaked gently during this period then washed with the same buffer solution until no protein was detected. Protein values were determined by the method of Lowry. The amount of unbound enzyme protein was subtracted from the total amount of enzyme protein used for immobilization, and the amount of bound enzyme protein was calculated as mg protein. g $carrier^{-1}$. Activities of GOD immobilized at different pH values were determined and optimal pH value for immobilization was revealed.

2.2.2. Immobilization temperature

GOD immobilization was carried out at temperatures 4, 10, 20, 30, 40 and 50 °C and at optimal pH determined before. For each case bound protein and catalytic activity of GOD were measured and optimal temperature for immobilization was determined.

2.2.3. Immobilization time and initial GOD concentration

GOD solutions at three different concentrations (0.5, 1.0 and 2.0 mg protein ml^{-1}) were prepared with a buffer solution of predetermined optimum pH. 2 ml of each GOD solution was added onto 0.2 g of activated carrier. Immobilization was carried out for 60, 90, 120, 150 and 180 min at optimal immobilization temperature. Bound enzyme amounts and catalytic activities for each case were determined.

2.3. Activity assay for free and immobilized GOD

Two methods were used to determine activity of GOD. One of them depends on the measurement of O_2 consumption by using an oxygenmeter (Lovibond Senso-direct), and the other depends on the measurement of the glucose concentration by DNSA method [24]. In the determination of GOD activity by using oxygenmeter, 10 ml of 100 mM β-D glucose solution, saturated with air and kept at room temperature at least 2 h for mutarotation, was used as substrate and residual oxygen concentration was measured. In the case of the DNSA method, 5 ml of 15 mM or appropriate concentration of β-D glucose saturated with air was used as substrate and reaction was started by adding free or immobilized GOD. Five milligrams of immobilized GOD or the free GOD solution containing the same amount of enzyme protein (5 mg) were used in the experiments. At the end of 10 min reaction time, 0.5 ml of reaction solution was added onto 0.5 ml of clear DNSA reagent. The resulting solution was kept in an boiling water bath for 10 min, and then immediately cooled in an ice bath for 1 min. Mixture was made up 8 ml by adding distilled water and absorbance was measured at 575 nm after 20 min. In order to provide a proper contact of substrate with immobilized GOD reaction mixture was gently agitated on a shaker.

2.4. Characterization of free and immobilized GOD

2.4.1. Effect of pH

Activities of free and immobilized GOD were determined at the same conditions in the different pH medium using glucose substrate prepared in 100 mM acetate buffer at pH 3.5, 4.0, 5.0, 5.5, 100 mM phosphate buffer at pH 6.0, 6.5, 7.0, 8.0 and 100 mM borate buffer at pH 9.0.

2.4.2. Effect of buffer concentration

Activities of free and immobilized GOD were determined for 100 mM glucose solution prepared by using 25, 50, 100, 150, 200 mM buffer solutions at predetermined optimal pH value.

2.4.3. Effect of temperature

The effect of temperature on the activity of free or immobilized GOD was investigated for the temperature range

of 10–60 °C at their optimal pH and buffer concentrations.

2.4.4. Kinetic parameters

The effect of substrate concentration (2–80 mM glucose) on the activities of free and immobilized GOD was investigated. Catalytic activities of free and immobilized GOD were investigated at their optimal conditions by using DNSA method. Michaelis–Menten coefficient (K_M) and maximum velocity (V_{max}) were determined from the Lineweaver–Burk plot.

2.4.5. Thermal stabilities of free and immobilized GOD

Free GOD solution containing 0.012 mg enzyme protein ml^{-1} was kept at 10, 20, 25, 30, 35, 40, 50 and 60 °C and residual activity was determined after 1, 3, 6, 10 and 24 h incubation times. The same type of measurements was carried out by using immobilized GOD samples incubated as dried solid forms.

2.4.6. Storage stabilities of free and immobilized GOD

Free GOD solution containing 0.012 mg enzyme protein ml^{-1} was kept at 5 °C or at 25 °C and residual activities of these samples were measured periodically for 2 months. The same type of measurements were carried out by using immobilized GOD samples incubated as dried solid forms.

2.4.7. Reusability of immobilized GOD

Reusability of immobilized GOD was investigated by using a batch type stirred column reactor as shown in Fig. 1. 50 mg immobilized enzyme was loaded into the reactor and 3 ml of 100 mM glucose solution at pH 6.0 was added into column, then reaction was allowed to continue for 5 min. Reaction mixture was removed immediately from the column

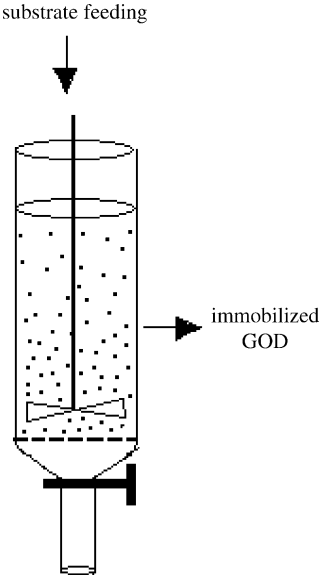

Fig. 1. Batch type stirred column reactor for investigation of reusability of immobilized GOD.

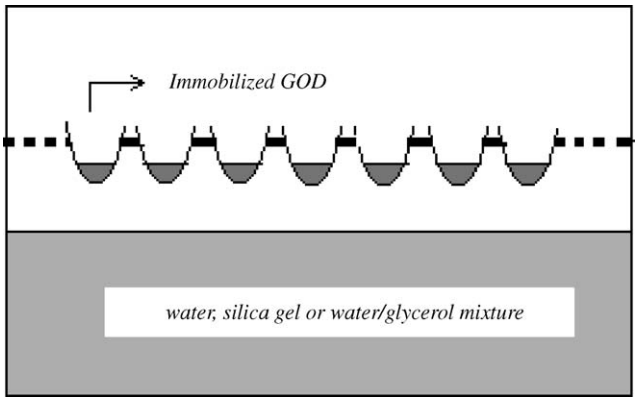

Fig. 2. The scheme of the systems used for the storage of the immobilized GOD in a medium with a chosen relative humidity.

and activity was determined by measuring the absorbance of H_2O_2 at 240 nm. Measurements were repeated 50 times by using the same enzyme reactor.

2.5. Storage stability of immobilized GOD in the mediums with different relative humidities

Immobilized GOD samples were stored in the mediums with different relative humidities at 25 °C. Storage mediums with different relative humidities were prepared by using appropriate ratios of water/glycerol mixtures to obtain 20, 40, 60 and 80% relative humidity. Silica gel was used to absorb medium moisture to obtain 0% relative humidity and only water used to provide 100% relative humidity. Immobilized GOD samples were put inside of the uncovered tubes (10 mm × 6 mm) and then tubes were placed into closed boxes of different relative humidity, Fig. 2. Each week water/glycerol solution of each box was changed with freshly prepared water/glycerol solution. Remaining activities of immobilized GOD stored in different storage mediums were determined periodically during 2 months.

3. Results and discussions

3.1. Optimization of the immobilization conditions

3.1.1. Effects of pH on GOD immobilization

To establish the optimum pH value for the immobilization of GOD onto florisil, pH of the immobilization medium was changed between 3.5 and 8.0. Fig. 3 shows mg of bound protein g $carrier^{-1}$ and the activity g $carrier^{-1}$ depending on immobilization pH. As seen in Fig. 3, the amount of bound protein was slightly affected from the medium pH in the range of 3.5–8.0. The maximum binding occurred at pH 4.2 which is the isoelectric point of GOD. At pH values greater than 4.2 the amounts of bound protein were steadily decreased with increasing pH up to 8.0. The low coupling efficiencies observed at pH values higher/lower than 4.2 may be explained by repulsive electrostatic forces occurred between already

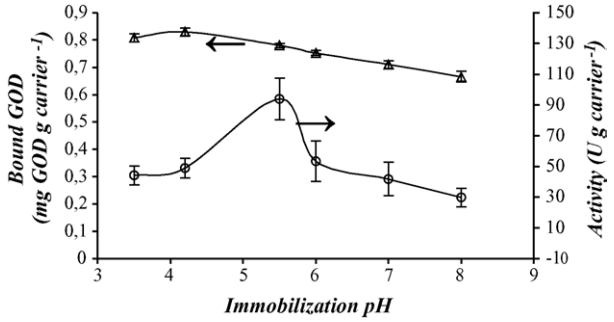

Fig. 3. Effects of immobilization pH on the amount of bound protein and activity of the immobilized GOD. (△) Bound GOD; (◯) activity of immobilized GOD.

bound GOD molecules onto florisil and unbound free GOD molecules having the same charge. This result was supported by the results of our another study which catalase (CAT) covalently immobilized onto florisil, too. In the case of CAT, the maximum coupling efficiency was found at pH 5.4 which is isoelectric point of CAT like GOD [25]. However, the maximum activity was measured for the GOD immobilized at pH 5.5. Our results clearly showed that the higher amount of immobilization of GOD on the carrier does not mean that it will exhibit the higher activity. So, subsequent immobilization studies were carried out at pH 5.5. Bulmuş et al. [10] reported the maximum amount of GOD immobilization onto modified polymethylmethacrilate (pMMA) at pH 4.0 but maximum activity was determined for GOD samples immobilized at pH 6.0.

3.1.2. Effect of temperature on GOD immobilization

Effect of temperature on GOD immobilization was investigated at different temperatures between 4 and 50 °C. It was observed that the amount of bound GOD and the activity of the immobilized samples were slightly affected from immobilization temperature. Maximum amount of GOD was bound at 10 °C as 0.765 mg protein g carrier^{-1} and this sample had also the highest catalytic activity. The amount of bound protein per grams of carrier was slightly decreased in the temperature range of 30–50 °C. This slight decrease may be related with increased mobility of GOD molecules which caused a decrease on adsorption of enzyme onto carrier. Considering the highest activity and maximum protein binding, we selected an immobilization temperature of 10 °C.

3.1.3. Immobilization time and initial GOD concentration

GOD solutions (10 ml) with three different initial GOD concentrations (0.5, 1.0 and 2.0 mg ml^{-1}) were used per unit weight of activated carrier and immobilizations were carried out at five different immobilization time. At the end of the tested immobilization time, each sample was washed with excessive buffer solution of pH 5.5. The amounts of the bound protein values for each sample were seen in Table 1. The amounts of bound protein were slightly increased with time until 150 min immobilization time and then a slight decrease was observed at 180 min for all three initial GOD concentrations. As shown from Table 1, the amount of bound GOD on the same amount of carrier was almost directly proportional with initial GOD concentration. Activities per gram carrier and specific activities of immobilized GODs were also given in Table 1. As we compared the results represented in Table 1, we concluded that the activity of immobilized GOD was not directly proportional with initial GOD concentration, in contrast to the amount of bound protein was directly proportional with initial GOD concentration. However, the maximum specific activity was obtained for immobilized GOD prepared with an initial GOD concentration of 0.5 mg ml^{-1} and at 120 min immobilization time. So, these values were accepted as the optimum immobilization conditions throughout the experiments.

3.2. Characterization of free and immobilized GOD

3.2.1. Effect of pH on activity

Effect of pH on the activities of free and immobilized GOD samples were investigated in the pH range 4.0–9.0 and results were given in Fig. 4. It was observed that the optimum pH of free enzyme was 5.5 while it was shifted to 6.0 for the immobilized GOD. We were expecting a considerable shift in optimal pH of immobilized GOD to less acidic region due to the production of the gluconic acid which may cause a decrease in the pH of the microenvironment of the immobilized enzyme. However, the deviation in the optimal pH was less than that we expect which may be explained with the basic property of florisil. Similar results were reported previously by Arıca et al. [26] and Yang et al. [27]. Activity of the immobilized GOD was less sensitive to pH changes at acidic pHs than alkaline pHs as compared with that of the free enzyme. Furthermore, it was observed that the immobilized

Table 1
The effects of initial GOD concentration and immobilization time on the amounts of bound GOD and activity of the immobilized GOD samples

Initial GOD concentration (mg ml^{-1})		The amounts of bound GOD (mg GOD g carrier^{-1})			GOD activity (U g carrier^{-1})			Specific GOD activity (U mg GOD^{-1})		
		0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0
Immbobilization time (min)	60	1.20	2.47	4.24	7.2 ± 0.7	10.7 ± 0.3	13.1 ± 1.2	6.0 ± 0.6	4.3 ± 0.1	3.1 ± 0.3
	90	1.48	3.15	6.06	8.3 ± 0.6	13.4 ± 1.3	19.0 ± 0.8	5.6 ± 0.4	4.2 ± 0.4	3.1 ± 0.1
	120	1.95	3.38	6.30	18.1 ± 1.1	19.8 ± 0.6	21.2 ± 0.3	9.5 ± 0.4	5.9 ± 0.7	3.4 ± 0.1
	150	1.98	3.90	7.59	8.2 ± 0.5	18.6 ± 0.6	25.9 ± 0.2	4.1 ± 0.3	4.8 ± 0.2	3.4 ± 0.0
	180	1.63	3.70	6.60	7.9 ± 0.4	14.8 ± 0.3	20.4 ± 0.1	4.8 ± 0.2	4.0 ± 0.1	3.1 ± 0.0

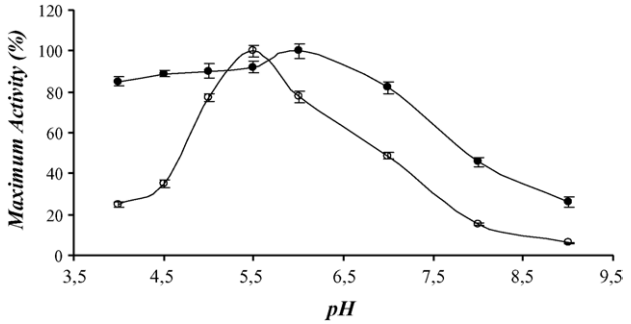

Fig. 4. Effect of the pH on the activity of the free and immobilized GOD. (◯) Free GOD and (●) immobilized GOD.

GOD was less affected from the medium pH than the free GOD.

3.2.2. Effect of buffer concentration on activity

Activities of free and immobilized GOD were measured at different buffer solutions with concentrations of 25, 50, 100, 150 and 200 mM at pH 5.5 for free and at pH: 6.0 for immobilized enzymes. The results were given in Fig. 5. In the reaction medium, pH is steadily decreased because of the gluconic acid production from the glucose oxidation by GOD. So, determination of optimal buffer concentration related with buffer capacity was very important. As seen from Fig. 5, activity of immobilized GOD was not significantly changed with buffer concentration in contrast with free enzyme. Free enzyme showed maximum activity at 100 mM buffer concentration, whereas immobilized GOD showed maximum activity at a smaller buffer concentration of 50 mM. It was clearly observed that immobilized GOD had much lower dependence on the buffer concentration than the free enzyme. At 200 mM buffer concentration free GOD lost most of its activity while immobilized GOD still kept it.

3.2.3. Effect of temperature on the activity

Activities of free and immobilized GOD samples were determined at different temperatures between 10 and 60 °C. The dependence of the both free and immobilized enzyme activities on temperature were found very similar. It was observed that, temperature affected the activity of the free and

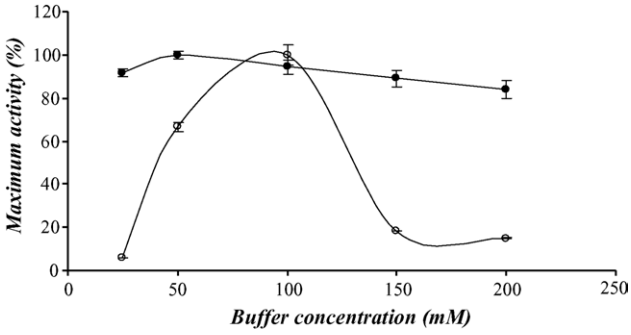

Fig. 5. Effect of the buffer concentration on the activity of the free and immobilized GOD. (◯) Free GOD and (●) immobilized GOD.

Table 2
Kinetic parameters of the free and immobilized GOD

GOD samples	V_{max} (μmol glucose dk^{-1} mg $protein^{-1}$)	K_M (mM)	E_a (kJ mol^{-1} K^{-1})	K_{cat}/K_M (s^{-1} M^{-1})
Free	435	68.2	32.8	1.7×10^4
Immobilized	217	259	45.2	2.2×10^3

immobilized GOD sharply and both had maximum activity at 35 °C. At 60 °C, the relative activities of free and immobilized GOD were found as 33% and 17% of their maximum activity, respectively. Gouda et al. [28] reported that the dissociation of FAD from free holoenzyme in aqueous medium was occurred at 59 °C and they concluded that dissociation of FAD from the holoenzyme was responsible for the thermal inactivation of GOD. The decrease in activity at high temperature for free and immobilized GOD may be related with the dissociation of FAD from holoenzyme. Limited solubility of O_2 in the reaction medium at high temperature may additionally affect the activity of the enzyme.

3.2.4. Effect of immobilization on kinetic constants

The effect of substrate concentration on the activities of the free and immobilized GOD was investigated. Experiments were conducted at predetermined optimal conditions. In these experiments, we used 0.012 mg free or 5 mg immobilized enzyme (containing 0.012 mg enzyme protein). The substrate (i.e. glucose) concentration was varied between 2 and 80 mM. Lineweaver- Burk plots used to obtain the K_M and V_{max} values. Catalytic efficiencies (k_{cat}/K_M) and activation energies (E_a) were also determined. K_M, V_{max}, E_a and k_{cat}/K_M values for both free and immobilized GOD were given in Table 2. As shown from Table 2, apparent K_M value of immobilized GOD was almost four times higher, and V_{max} value was two times lower than those of free enzyme. The higher K_M values for the solid-phase enzymes may be result of a number of effects. The migration of substrate from the solution to the microenvironment of an immobilized enzyme can be a major factor that cause an increase in K_M value [29].

In general, apparent K_M values of immobilized enzymes are higher, and V_{max} values are lower than those for free enzymes, mainly due to diffusion limitations and steric hindrances in the immobilized forms. This opinion was supported by the results of experimental studies reported earlier [8,10,15,27]. It may be the results of these changes, catalytic efficiency of immobilized enzyme (2.2×10^3 s^{-1} M^{-1}) was 7.7-fold smaller than that of free GOD (1.7×10^4 s^{-1} M^{-1}) in our study.

The activation energies of free and immobilized GOD were 32.8 and 45.2 k J mol^{-1}, respectively.

3.2.5. Thermal stabilities of free and immobilized GOD

Thermal stabilities of free and immobilized GOD samples were investigated at eight different temperatures (10, 20, 25, 30, 35, 40, 50 and 60 °C) for five different preincubation times (1, 3, 6, 10 and 24 h). It was observed that at temperatures

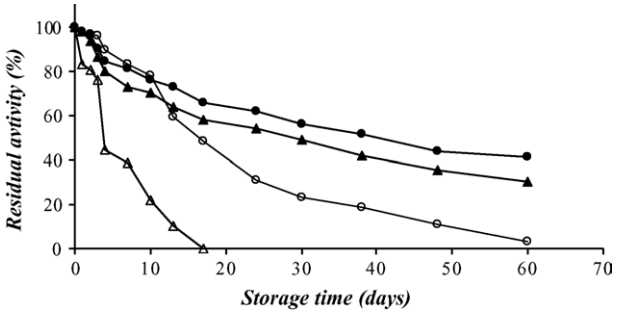

Fig. 6. Storage stability of the free and immobilized GOD. (●) Immobilized GOD at 4 °C; (◯) free GOD at 4 °C; (▲) immobilized GOD at 25 °C; and (△) free GOD at 25 °C.

between 10 and 40 °C, free GOD retained its initial catalytic activity better than immobilized GOD. Remaining activities of free and immobilized GODs were similar at 50 °C. At 60 °C, activity of free GOD decreased sharply and at the end of 24 h incubation time no activity was measured. In the case of the immobilized GOD, the decrease in the activity was not sharp and at the end of 24 h incubation time enzyme still had some activity even it was so small. This was related to incubation of immobilized GOD as dried solid form which may contribute the prevention of the dissociation of FAD from holoenzyme.

3.2.6. Storage stabilities of the free and immobilized GOD

As shown from Fig. 6, the immobilized GOD samples stored at 5 °C and 25 °C were both more stable than the free GOD samples stored at the same temperatures. Free enzyme when stored at 25 °C lost its all activity at the end of the 17 days while immobilized GODs stored at 4 and 25 °C saved 41.1% and 30.2% of their initial activities, respectively. Ying et al. [30] investigated the storage stability of GOD immobilized on micro porous membranes prepared from poly(vinylidene fluoride) with grafted poly(acrylic acid) side chains. They reported that free and immobilized GOD stored in the phosphate buffer (pH 7.4) at 4 °C and saved 35% and 55% of their initial activity. Li et al. [15] immobilized GOD on the surface of polyaniline films, investigated the storage stability of the free and immobilized GOD samples and reported that immobilized GOD still retained about 65% of its original activity in the phosphate buffer solution (pH: 7.4) over a period of 2 months whereas free enzyme saved only 40% of its initial activity.

3.2.7. Reusability of immobilized GOD

Reusability of immobilized GOD was investigated by using the stirred batch type column reactor using the same enzyme preparation for 50 times. There was about 20 s between two following cycles. In order to prevent the influence of storage time on the enzyme activity, 50 measurements were carried out at the same day. Activities related with reuse numbers were given in Fig. 7. After five reuses, residual activity was about 80% of the initial activity. Immobilized GOD retained approximately 40% of its initial activity at the end of 50 uses. Tzanov et al. [4] reported that covalently immobilized GOD onto alumina support was reusable for at least three cycles each of them with a duration of 7.5 h, without significant loss in activity.

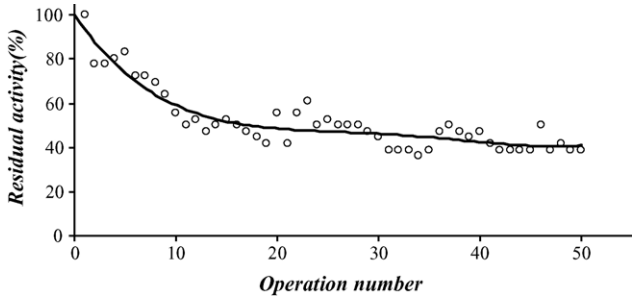

Fig. 7. Operational stability of the immobilized GOD.

3.3. Stability of immobilized GOD stored under different relative humidity

Immobilized GOD samples were stored in different mediums of 0–100% relative humidity for 2 months. Residual activities of these samples were determined periodically and results were presented in Fig. 8. It was clearly shown that the stabilities of the immobilized GODs stored in the medium of 60% relative humidity, were maximum for all measurements during 2 months and the residual activities of these samples were unexpectedly greater than the initial activities during 22 storage days. The stability of the immobilized GOD stored in the medium of 0% relative humidity was minimum for all measurements during 2 months. Immobilized GOD sample, stored in a medium 0% relative humidity lost almost 50% of its initial activity at the end of the first storage day and 83% of its initial activity at the end of the 60 storage days. Interestingly, the residual activities of the other immobilized

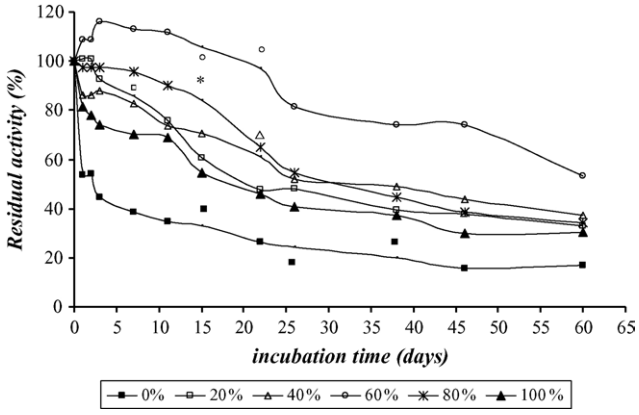

Fig. 8. Storage stabilities of the immobilized GOD stored in the mediums of different relative humidity. The relative humidities of the mediums were represented as (■) 0%; (□) 20%; (△) 40%; (◯) 60%; (●) 80%; and (▲) 100%.

GOD samples stored in the mediums with relative humidity of 20, 40, 80 and 100% were in between the residual activities of the samples stored in the mediums with relative humidity of 0 and 60%. At the end of the 60 days storage period, remaining activities of immobilized GOD samples stored in the mediums of 0, 20, 40, 60, 80 and 100% relative humidity were 17.0, 32.8, 37.1, 53.4, 34.0 and 30.3%, respectively. It was shown that, the storage stability of the immobilized GOD sample increased with relative humidity from 0 to 60% however, stability decreased for the higher (80–100% relative humidity) values.

4. Conclusion

The catalytic efficiency of the immobilized GOD was about 17% of the catalytic efficiency of the free enzyme and the decrease in catalytic efficiency may be due to immobilized GOD inactivation from H_2O_2 produced in reaction medium. However, immobilized GOD was less sensitive to changes in both buffer concentrations and pHs than the free GOD. This may provide the use of immobilized GOD in a broad range of experimental pH and buffer concentrations. Immobilized GOD has greater storage and operational stability which may considered as another advantages of its use in possible industrial applications. The medium with relative humidity of 60% may be proposed as a suitable storage medium for immobilized GOD onto florisil.

Acknowledgements

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