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Bioconversion of D-glucose into D-glucosone by immobilized glucose 2-oxidase from *Coriolus versicolor* at moderate pressures

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ABSTRACT

The immobilized glucose 2-oxidase (pyranose oxidase, pyranose:oxygen-2-oxidoreductase, EC 1.1.3.10) from *Coriolus versicolor* was used to convert D-glucose into D-glucosone at moderate pressures, up to 150 bar, with compressed air in a modified commercial batch reactor. Several parameters affecting biocatalysis at moderate pressures were investigated as follows: pressure, different forms of immobilized biocatalysts, glucose concentration, pH, temperature and the presence of catalase. Glucose 2-oxidase (GOX2) was purified by immobilized metal affinity chromatography on epoxy-activated Sepharose 6B-IDA-Cu(II) column at pH 6.0. Purified enzyme and catalase were immobilized into a polyethersulfone (PES) membrane in the presence of glutaraldehyde and gelatin. Enhancement of the bioconversion of D-glucose was done by the pressure since an increase in the pressure with compressed air increases the conversion rates. The optimum temperature and pH for bioconversion of D-glucose were found to be 62 °C and pH 6.0, respectively and the activation energy (E_a) was 28.01 kJ mol^{-1}. The apparent kinetic constants (V'_{max}, K'_m, K'_{cat} and K_{cat}/K'_m) for this bioconversion were 2.27 U mg^{-1} protein, 11.15 mM, 8.33 s^{-1} and 747.38 s^{-1} M^{-1}, respectively. The immobilized biomass of *C. versicolor* as well as crude extract containing GOX2 activity were also useful for bioconversion of D-glucose at 65 bar with a yield of 69.9 ± 3.8% and 91.3 ± 1.2%, respectively. The immobilized enzyme was apparently stable for several months without any significant loss of enzyme activity. On the other hand, this immobilized enzyme was also stable at moderate pressures, since such pressures did not affect significantly the enzyme activity.

1. Introduction

Enzymes are ideal biocatalysts for stereo- and regioselective reactions which take place in aqueous medium under mild conditions [1]. Fungal glucose 2-oxidase (GOX2, pyranose oxidase, pyranose:oxygen-2-oxidoreductase, EC 1.1.3.10) has been used to catalyse the oxidation of D-glucose at C-2 position producing 2-keto-D-glucose (D-glucosone, D-arabino-hexos-2-ulose) which is an important precursor for biosynthesis of the antibiotic cortalcerone [2]. Moreover, this enzyme also exhibits activity over other carbohydrates such as D-galactose, L-sorbose and 1-deoxy-D-glucose producing the corresponding keto-sugars [3]. Therefore, dicarbonyl sugars are widely used for synthesis of rare sugars such as antibiotics, pyrrolidine and piperidine aminosugars. However, the bioconversion of D-glucose and other carbohydrates in the presence of GOX2 and catalase into their corresponding keto-sugars is very slow and time-consuming at atmospheric pressure [4,5]. Moreover, GOX2 activity from basidiomycete strains (i.e. *Coriolus versicolor* and *Phanerochaete chrysosporium*) is highly unstable [6,7] and there is a great need to stabilize the enzyme activity by several ways such as by protein engineering and by immobilization in a suitable support [8].

Immobilization of enzymes to solid carriers is the most widely used strategy to improve stability of biocatalysts such as storage and operational stabilities. Moreover, this strategy can increase selectivity towards other substrates compared with the corresponding free enzymes. The four main methods of immobilization are as follows: (a) adsorption, (b) covalent binding, (c) entrapment and (d) membrane confinement. In general, thermal stability of biocatalyst is due to the molecular rigidity introduced by binding to a rigid support and creation of a protected microenvironment for the biocatalyst [9,10].

Enzyme reactions can be carried out under high pressure (i.e. over 2 kbar) or in supercritical fluids which is a new and promising field of enzyme engineering [11]. High pressure is responsible for direct conformational changes in enzymes which affect their biological activities [12,13]. However, such high pressures may inactivate some enzymes since undesirable changes may occur in

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enzyme conformation which results in enzyme inactivation [13]. Moreover, pressure also influences the reaction rate constant which changes according to transition state theory and standard thermodynamics [14]. There are many reports in the literature on the effect of high pressure (i.e. over 2 kbar) on enzyme structure and activity [12,15]. However, few published work have been found in the literature regarding the use of moderate pressures, in the range of 1–150 bar, on enzyme structure and activity. Therefore, the present work is concerned with the use of moderate pressure up to 150 bar by compressed air in a pressure batch reactor in order to increase the rate of bioconversion of D-glucose since oxygen is one of the substrates of the reaction catalysed by immobilized GOX2. Several parameters affecting this bioconversion will be investigated such as pressure, different forms of biocatalysts, D-glucose concentration, pH, temperature and the presence of catalase.

2. Materials and methods

2.1. Materials

C. versicolor was isolated from old growth forest of Olympic Peninsula (Port Townsend, Washington, USA). Corn-steep liquor was kindly donated by COPAM, Portugal. O-dianisidine, iminodiacetic acid, D-glucose, D-glucosone, 1,4-butanediol diglycydil ether, glutaraldehyde, catalase and peroxidase were purchased from Sigma Chemical Company (USA). Sepharose 6B was obtained from GE Health Care, Sweden.

Sugar Pak column was obtained from Waters (USA) and discs of 3.0 cm diameter of modified polyethersulfone (PES) membranes (Ultrabind, US450 0.45 μm) were purchased from Pall Gelmam Laboratories (USA).

2.2. Enzyme production and purification

GOX2 from *C. versicolor* was produced by submerged fermentation by using a basal culture medium containing agro-industrial wastes as carbon sources such as whey powder (2.5%) and other components as follows: corn-steep liquor (0.7%), $MgSO_4$ (0.15%) $CaCl_2$ (0.0125%) and KH_2PO_4 (0.1%). They were all dissolved in tap water which also contained trace amounts of salts as follows: 1.0 μM $CuSO_4$, 1.0 mM $CaCO_3$, 0.06 mM $MgCl_2$, 0.9 μM $AlCl_3$, 1.3 μM $MnCl_2$, 0.01 mM $NaNO_3$, 0.85 μM NaF, 1.7 μM $FeCl_3$, 1.1 μM ZnCl and 0.1 mM NaCl. The pH of the medium was adjusted to 5.5 and sterilized at 121 °C for 20 min in an autoclave. Alternatively, other agro-industrial wastes could be used such as tomato pomace and rice bran.

Erleynmeyer flasks (500 ml) containing 100 ml of sterile medium were inoculated with two pieces of 0.5 cm × 0.5 cm plugs of the appropriate culture grown in petri dishes and the culture was grown at 200 rpm, 25 °C for several days. By this time it was possible to detect GOX2 activity in the biomass. This inoculum was used to inoculate batch fermenter (2.5 l) containing the same culture medium (2.0 l) and the culture was grown at 200 rpm, pH 5.5 and 25 °C for several days and GOX2 production was followed as a function of time. Aliquots (10 ml) were removed daily from the fermenter under aseptic conditions; the biomass was recovered by filtration and washed with saline. The biomass was resuspended in 2 volumes of 50 mM phosphate buffer pH 6.5 and sonicated at 100 W for 2 min in an ice bath. The suspension was centrifuged at 10,000 × *g* for 5 min and the cell-free supernatant was the source of GOX2 activity. The enzyme was purified by immobilized metal affinity chromatography on epoxy-activated Sepharose 6B-IDA-Cu (II) as described previously [16].

2.3. Electrophoretic analysis

Crude extract and purified enzyme were analysed for purity by SDS-PAGE and native PAGE as reported previously [17,18].

2.4. Enzyme assays

For routine enzyme assays in fungal cultures and chromatographic fractions, GOX2 activity at atmospheric pressure was determined by using o-dianisidine, D-glucose (0.1 M) and peroxidase (1 U) in 50 mM phosphate buffer pH 6.5. The reaction mixture contained 0.79 ml of o-dianisidine (6 mg/100 ml), 0.01 ml peroxidase (1 mg ml^{-1}), 0.1 ml glucose (1 M) and 0.1 ml of crude extract. After 10 min, the absorbance was recorded at 450 nm at 25 °C ($\varepsilon = 8.3 \times 10^3$ cm^{-1} M^{-1}). Alternatively, enzyme activity was assayed at atmospheric pressure by using 0.1 ml of appropriate concentration of D-glucose in 50 mM phosphate buffer pH 6.0 and suitable amount of either crude extract or purified enzyme in a final volume of 1 ml by bubbling with air. Aliquots were removed from the reaction mixture at suitable time intervals which were analysed by HPLC in terms of substrate and product as described below. Catalase activity was assayed in the presence of H_2O_2 as reported previously [16].

2.5. Protein assay

Protein concentration was determined by Coomassie blue dye binding method [19].

2.6. Immobilization of GOX2 and catalase, crude extract and biomass of *C. versicolor*

Either crude extract containing GOX2 activity or purified enzyme and catalase were immobilized by covalent bonding in PES membranes (discs of 3.0 cm diameter) by using the following reaction mixture: 80 U of purified GOX2, 10 mg of catalase (1000 U mg^{-1} protein), 2 μl of glutaraldehyde (5%, v/v) and 20 μl gelatin (25%) in 50 mM phosphate buffer pH 6.0 in a final volume of 200 μl. The mixture was immediately transferred to membrane discs which were dried and washed several times with 50 mM phosphate buffer pH 6.0 [20]. In some experiments, GOX2 was immobilized on PES membranes without catalase by using the methodology described above. Alternatively, crude extract (0.21 U mg^{-1} protein) was immobilized in PES membranes by using the following reaction mixture: crude enzyme having 80 U of activity, 10 mg catalase (1000 U mg^{-1} protein), 10 μl of glutaraldehyde (5%, v/v) and 50 μl gelatin (25%) as described above. Regarding biomass immobilization, 1.0 g (wet weight) of biomass of *C. versicolor* having 80 U of enzyme activity, was mixed with 1.0 ml of glutaraldehyde (5%, v/v) and 1.0 ml gelatin (25%) in 50 mM phosphate buffer pH 6.0. The biomass was allowed to dry, washed with 50 mM phosphate buffer pH 6.0 and subsequently it was wrapped in a dialysis membrane (P10) for biocatalysis in a batch reactor. Alternatively, biomass of *C. versicolor* was wrapped in a dialysis membrane (P10) and used for biocatalysis. All immobilized forms of biocatalysts were stored at 4 °C in 50 mM phosphate buffer pH 6.0.

2.7. Biocatalysis at moderate pressure

2.7.1. Apparatus set-up

The experimental set-up consists basically of one batch reactor (Micro Reactor Parr Instruments CO, 4843) with 25 ml of capacity equipped with agitation, temperature and relative pressure reading devices. Two principal valves inlet and outlet connections and a rupture disk set a 250 bar were used to perform the enzyme reactions. In each experiment samples were withdrawn at appropriate intervals to determine product concentration by using an HPLC system (Jasco Instruments).

Biocatalysis at moderate pressure was carried out by using either immobilized crude extract and catalase (0.05 U mg^{-1} protein) in PES membranes, immobilized purified enzyme and catalase in membranes (1.5 U mg^{-1} protein), biomass wrapped in a dialysis membrane or immobilized biomass from *C. versicolor* (1.0 g) wrapped in a dialysis membrane. The reaction mixture contained appropriate concentration of D-glucose in 50 mM phosphate buffer pH 6.5 (19.0 ml) and appropriate form of enzyme which was stirred at 110 rpm at 23 °C. The reaction was performed at several relative pressures at 23 °C by using compressed air. Aliquots (100 μl) were removed from the batch reactor at suitable time intervals and the substrate and product of the reaction were analysed by HPLC as described below.

2.7.2. Calibration curve for D-glucose and D-glucosone

Several concentrations of D-glucose and its corresponding keto-sugar were prepared in 50 mM phosphate buffer pH 6.5. These samples were analysed in an HPLC system (Jasco) by using a Sugar Pak column at 75 psi, 90 °C, 0.1 mM Ca(II) EDTA in millipore water as the solvent and at a flow rate of 0.5 ml min^{-1}.

2.8. Assay of GOX2 by HPLC analysis

Samples (25 μl) from reaction mixtures were injected into the HPLC system and the chromatograms were obtained in 12 min time since the retention time of D-glucose and D-glucosone were 9.3 and 10.3 min, respectively. The peak area was determined as a function of either D-glucose or D-glucosone concentration.

2.9. Kinetic characterization of purified preparation of immobilized GOX2 and catalase at moderate pressures

Immobilized GOX2 activity containing co-immobilized catalase in PES membranes was used throughout these kinetic studies. It was assayed by using D-glucose as the substrate and one unit of GOX2 activity was defined as the amount of enzyme required to oxidize 1 (mol substrate per min. under the same experimental condition. Assays in pressure batch reactor were performed at suitable moderate pressures, up to 120 bar, in 50 mM of the appropriate buffer and pH, and suitable aliquots were removed from the pressure reactor at appropriate time intervals and analysed by HPLC as described above. The optimum pH of GOX2 activity was investigated at 110 bar by using purified immobilized enzyme preparation in a reaction mixture containing 0.1 M D-glucose in several buffers as follows: 50 mM citrate pH 4.6 and 5.6, 50 mM phosphate buffer pH 6.0 and 7.0 and 50 mM Tris pH 7.0 and 8.0. The optimum temperature of GOX2 activity was investigated by using purified immobilized enzyme preparations at 110 bar in a reaction mixture containing 100 mM D-glucose in 50 mM phosphate buffer pH 6.0 which was incubated at different temperatures (i.e. 25, 35, 45, 55, 62, 66, 70 and 80 °C) for 5 min. The activation energy for immobilized GOX2 reaction was determined by measuring the enzyme

activity at different temperatures and transforming them according to the Arrhenius equation:

$$\mathrm{Ln}(v) = A - \frac{E_a}{R} \times \frac{1}{T}$$

where v is reaction rate; T is the absolute temperature (K), E_a is the activation energy and R is gas constant.

The apparent kinetic constants (V'_{max}, K'_m, K'_{cat} and K_{cat}/K'_m) for D-glucose were determined by Michaelis–Menten plot by using the software from Sigma Plot 2004 (version 9.01).

2.10. Biocatalysis at atmospheric pressure

For comparative purposes, biocatalysis at atmospheric pressure, was also carried out by using either immobilized cell-free extract and catalase in PES membranes (0.05 U mg^{-1} protein), immobilized biomass of *C. versicolor* (1.0 g wet weight) and purified immobilized enzyme and catalase in PES membranes (1.5 U mg^{-1} protein). The reaction mixture contained appropriate concentration of D-glucose in 50 mM phosphate buffer pH 6.5 (19.0 ml) and appropriate form of enzyme/biocatalyst which was stirred at 110 rpm in a magnetic stirrer. The reaction was performed by using the reaction mixture bubbled with air ([O_2] = 8.89 mg l^{-1}). The solubility of O_2 in the solution is assumed as in fresh water, using the toolbox and results from [21] and the respective corrections when O_2 is pure against the concentration in the air.

2.11. Stability of immobilized GOX2 and catalase at moderate pressures

Membrane preparations containing immobilized GOX2 and catalase were stored in 50 mM phosphate buffer pH 6.0 at 4 °C and assayed for activity in a pressure batch reactor at 100 bar, 110 rpm at 23 °C in a reaction mixture containing 0.1 M D-glucose in 50 mM phosphate buffer pH 6.0 as described above. Such membranes were assayed at appropriate time intervals for several months in order to investigate its stability. Alternatively, PES membrane preparations containing immobilized GOX2 were incubated 50 mM phosphate buffer pH 6.0 (20 ml) in batch reactor at 70 bar, 100 rpm at 23 °C for several days. Membrane preparations were removed at suitable time intervals and immobilized GOX2 activity was measured at atmospheric pressure by HPLC. For comparative purposes, the stability of the immobilized enzymes (i.e. GOX2 and catalase) in PES membranes was also investigated at atmospheric pressure for several days at 100 rpm in a magnetic stirrer at 23 °C. Membrane preparations were removed at suitable time intervals and immobilized GOX2 activity was measured by HPLC.

3. Results and discussion

3.1. Biocatalysis at moderate and atmospheric pressures

The calibration curves for D-glucose and D-glucosone were carried out by HPLC [22] which exhibited linear relationship between the peak area (mV) and either D-glucose or D-glucosone concentration (data not shown).

The immobilization of GOX2 and catalase on PES membranes revealed that the recovery of immobilized enzyme activity was 14.1 ± 0.92% and 11.5 ± 1.01%, respectively (data not shown). The amount of GOX2 immobilized on PES membranes was found to be 5.1 mg which was determined by the indirect method (i.e. Coomassie blue dye binding method) because extensive washing of PES membrane was not required. This protein value is five-fold higher than the manufacturer data for this membrane in the absence of glutaraldehyde. However, in the present work the enzyme was immobilized in the presence of glutaraldehyde which may increase the membrane capacity.

The formation of D-glucosone and the consumption of D-glucose were followed by HPLC when the reaction was carried out at atmospheric pressure and batch reactor by using either purified immobilized enzyme and catalase or purified soluble enzyme and catalase (Fig. 1). These data revealed that the enzyme reaction is very slow at atmospheric pressure (Fig. 1), compared with the reaction in a batch reactor (i.e. 70 bar, [O_2] = 2960.0 mg l^{-1}) (Fig. 1). This result may be explained on the basis that molecular O_2 is one of the substrates of this enzyme reaction and its concentration is much higher in batch reactor at 70 bar compared with atmospheric pressure ([O_2] = 8.89 mg l^{-1}). However, the activating effect of moderate pressures on immobilized GOX2 activity cannot be ruled out since

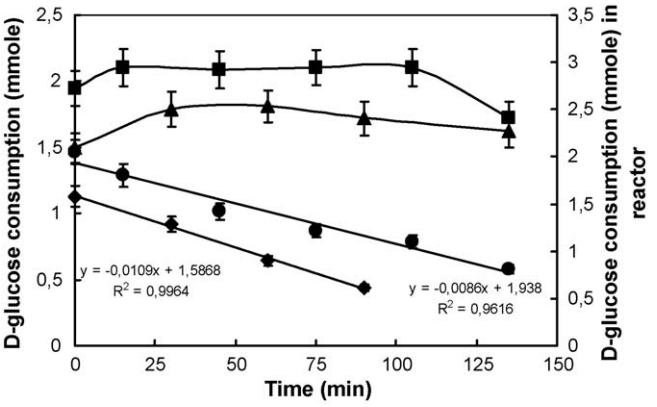

Fig. 1. Progress curve of consumption of D-glucose by free and immobilized GOX2. Atmospheric pressure: immobilized GOX2 and catalase (■) and free GOX2 (9.0 U) and catalase (10 mg) (▲); 72 bar: immobilized form (♦) and free form (●).

several reports in the literature have described this effect on several enzyme reactions [12,23]. The oxidation of D-glucose in the presence of immobilized GOX2 and catalase was also carried out at several working pressures at room temperature as shown in Fig. 2A. These data suggest that by increasing the pressure there is an increase in the conversion of D-glucose compared with atmospheric pressure because [O_2] is much higher in batch reactor than at atmospheric pressure. Several experiments were carried out by increasing the pressure up to 160 bar in the batch reactor which exhibited an increase in rate of reaction catalysed by immobilized GOX2 and catalase (data not shown). Furthermore, the conversion of D-glucose into D-glucosone was investigated by using different enzyme preparations such as immobilized crude extract and immobilized biomass from *C. versicolor*, either in a batch reactor or at atmospheric pressure (Fig. 2B). The data presented in Fig. 2B revealed that crude extract exhibited a higher degree of conversion compared with purified enzyme preparation by using the same amount of enzyme activity. Surprisingly, the biomass of *C. versicolor* containing GOX2 activity was also useful for conversion of D-glucose into D-glucosone at moderate pressure. This rather low degree of bioconversion for the biomass compared with the crude extract may be due to mass transfer limitations (Fig. 2B) which has been reported by several researchers [24,25]. The aim of this experiment was to show that it was possible to immobilize several forms of biocatalysts and use them in batch reactor for biocatalysis either at moderate or atmospheric pressures. However, the comparative analysis of these biocatalysts is difficult because: 1. the amount of glutaraldehyde used for each biocatalyst was different since the total amount of protein in each case was also different; 2. the microenvironment as well as steric hindrance of each immobilized biocatalyst in PES membrane is different; 3. mass transfer limitations for each immobilized biocatalyst are also different and 4. the amount of immobilized enzyme may be different for each biocatalyst. Biocatalysis at atmospheric pressure revealed a low degree of D-glucose bioconversion with different immobilized biocatalysts compared with the batch reactor (Fig. 2B). Immobilized biomass of *C. versicolor* could be used several times for bioconversion of D-glucose and about 2/3 of GOX2 activity was lost after the 10th re-utilization of immobilized biomass in batch reactor (Fig. 2C). In fact, the data in Fig. 2C revealed that the initial velocity in the 1st use of biomass was 0.507 mmol D-glucosone h^{-1} whereas the initial velocity in the 10th re-use of biomass was 0.156 mmol h^{-1}. Therefore, after 10th re-utilization of biomass, the initial velocity was 30.77% of the 1st use of biomass. Moreover, the yield of D-glucosone from D-glucose by using either the immobilized biomass of *C. versicolor* or immobilized crude extract was found to be 69.9 ± 3.8% and

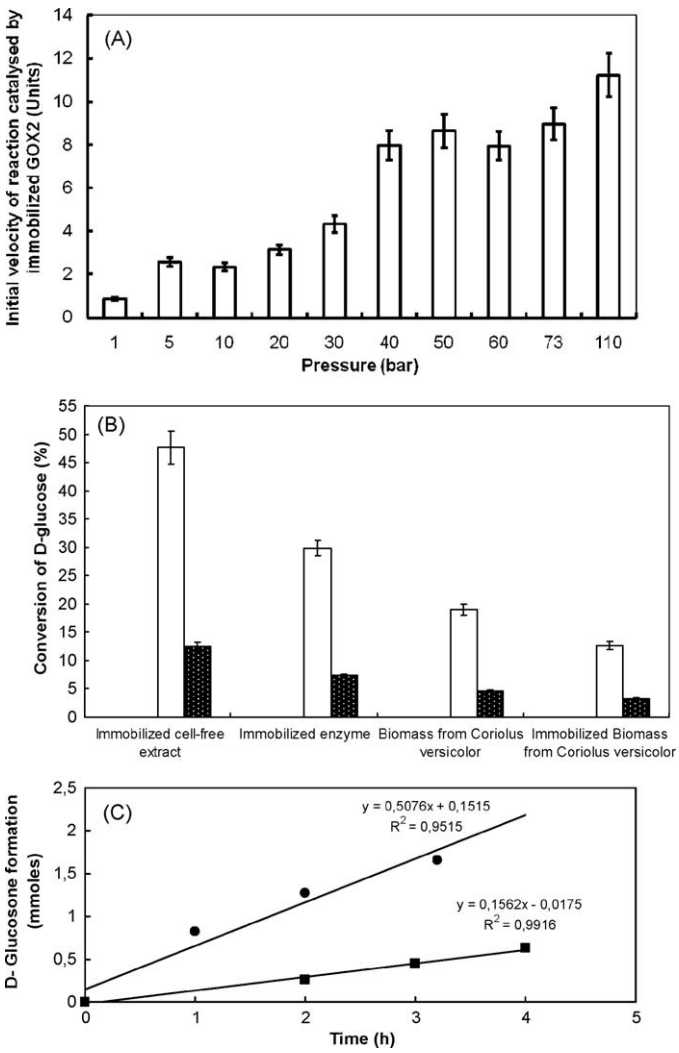

Fig. 2. (A) The effect of pressure on bioconversion of D-glucose by immobilized GOX2. The reaction at atmospheric pressure was carried out at 23 °C by bubbling the reaction mixture with air. (B) Bioconversion of D-glucose by different forms of biocatalysts by applying 70 bar of pressure (white bar) and without pressure (dark bar). (C) The reusability of immobilized biomass from *C. versicolor* on the rate of bioconversion of D-glucose at 65 bar at 1st use of biomass (●) and 10th re-utilization of biomass (■).

91.3 ± 1.2%, respectively (data not shown). In addition, the presence of catalase in PES membranes increased significantly the degree of conversion of D-glucose (data not shown) suggesting that H_2O_2 acts as a powerful inhibitor for this enzyme which is in agreement with published reports [6].

3.2. Kinetic characterization of immobilized GOX2 from C. versicolor at moderate pressures

The bioconversion of D-glucose in the presence of immobilized GOX2 and catalase was carried out at several working temperatures at moderate pressures as shown in Fig. 3A. These data suggest that optimum temperature for maximum conversion of D-glucose into D-glucosone under these experimental conditions is 62 °C which is higher than the value of 50 °C reported for the free enzyme at atmospheric pressure [26]. From the linear range of the Arrhenius plot, the activation energy (E_a) was determined to be 28.01 kJ mol^{-1} which is lower than the value of 34.6 kJ mol^{-1} reported for this soluble enzyme at atmospheric pressure [4]. There are few reports on immobilization of GOX2 for biocatalysis in the literature [10,27–29]

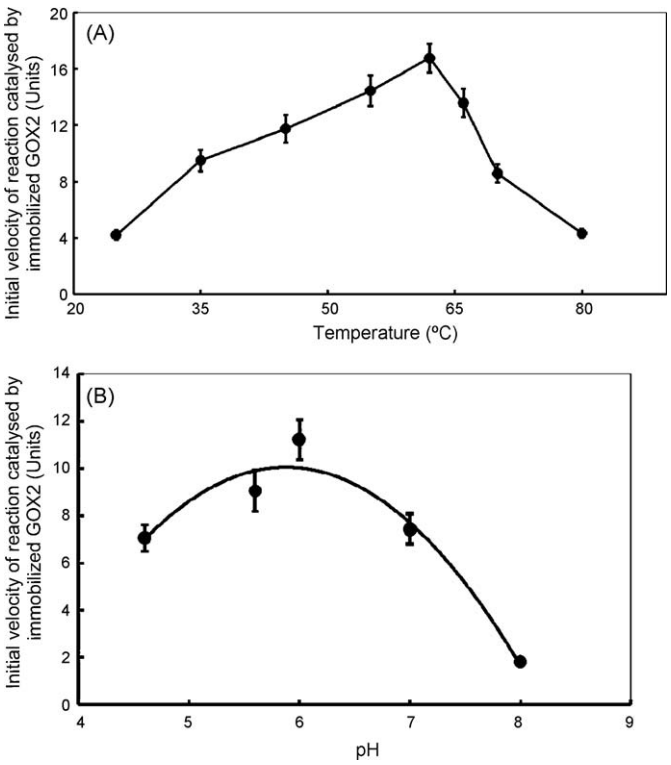

Fig. 3. The effect of temperature and pH on the initial velocity of the reaction catalysed by immobilized GOX2 and catalase at 110 bar. (A) Temperature and (B) pH.

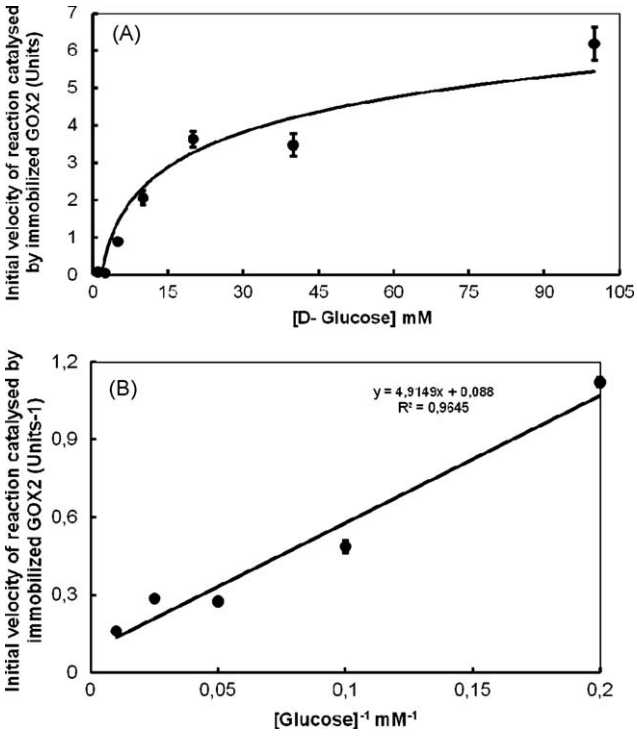

Fig. 4. The effect of D-glucose concentration on the initial velocity of the reaction catalysed by immobilized GOX2 and catalase at pH 6.0 and 110 bar. Michaelis–Menten (A) and Lineweaver–Burk (B) plots.

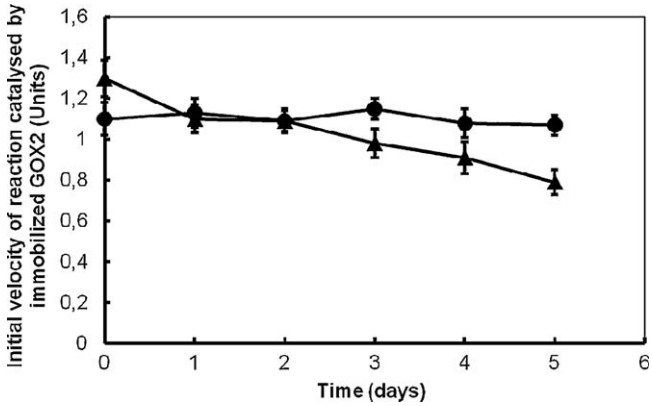

Fig. 5. Stability of immobilized GOX2 at moderate pressures. Several PES membranes containing GOX2 and catalase (1.2 U) were incubated in 50 mM phosphate buffer pH 6.0 either in batch reactor at 70 bar (●) or at atmospheric pressure (■).

which have described that there are no differences in optimum temperature of activity of the free and immobilized forms of this enzyme [29]. The effect of pH on the bioconversion of D-glucose into D-glucosone at moderate pressure was also investigated which revealed that the optimum pH is 6.0 for maximum conversion of D-glucose into D-glucosone by using immobilized GOX2 and catalase (Fig. 3B). These data are in agreement with the pH value of 6.2 reported for this soluble enzyme at atmospheric pressure [3,26].

The effect of D-glucose on initial velocity of immobilized GOX2-catalysed reaction at moderate pressures of 110 bar was carried out by using PES membrane containing GOX2 and catalase (Fig. 4). The apparent kinetic constants (i.e. V'_{max}, K'_m, K'_{cat} and K_{cat}/K'_m) were determined by using Sigma Plot which were found to be 2.27 U mg^{-1} protein, 11.15 mM, 8.33 s^{-1} and 747.38 s^{-1} M^{-1}, respectively (Fig. 4). These results are lower than the data reported in the literature for soluble enzyme preparations which may be due to mass transfer limitations as well as the use of different assay conditions for this enzyme since linked enzyme assays with peroxidase were used in such kinetic studies [24–26,29].

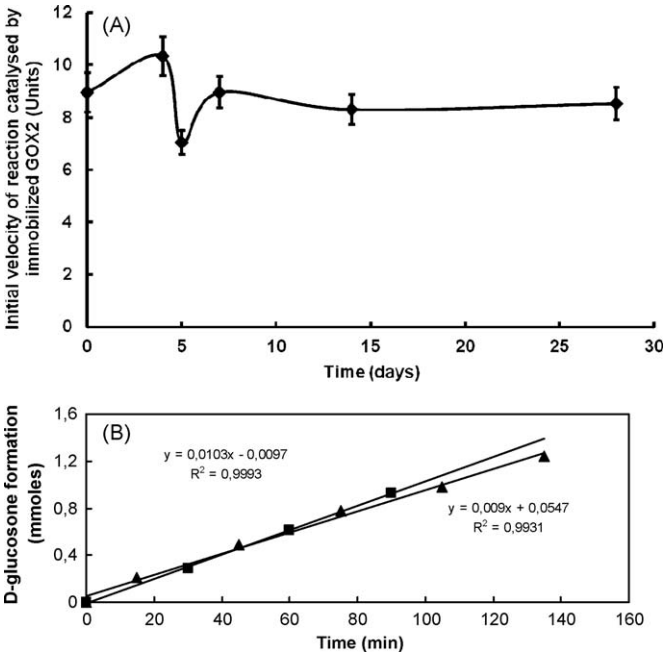

Fig. 6. Storage stability of immobilized GOX2. A PES membrane was stored in 50 mM phosphate buffer pH 6.0. (A) The membrane was assayed for activity at suitable time intervals at 110 bar. (B) Initial velocity of the reaction catalysed by immobilized GOX2 on the 4th day (■) and on the 75th day (▲) at 110 bar.

3.3. Storage stability of immobilized GOX2 at moderate pressures

The effect of moderate pressures on the stability of immobilized GOX2 activity was studied by incubating several preparations of immobilized enzyme in batch reactor for several days. The data presented in Fig. 5 revealed that it did not affect significantly the enzyme activity at 70 bar compared with the enzyme stored at atmospheric pressure. In addition, the storage stability of immobilized GOX2 on PES membranes containing catalase was also investigated which revealed that this enzyme preparation was apparently stable for several months without any significant loss of enzyme activity (Fig. 6). The operational stability of this immobilized enzyme was studied at moderate pressures which revealed that it was stable for at least 30 days without any substantial loss of GOX2 activity (data not shown). On the other hand, this is also the first report on the use of either immobilized crude extract or biomass from *C. versicolor* for bioconversion of D-glucose at moderate pressures. Such membrane supports are highly suitable for immobilization of GOX2 which exhibit high stability for several months.

4. Conclusions

The data presented in this work have revealed that at moderate pressure, there are several parameters that affect the degree of conversion of D-glucose into D-glucosone namely, temperature, pH, different forms of immobilized biocatalysts and the presence of catalase. The optimum pH and temperature for this reaction at moderate pressures were slightly different compared with the data published for this enzyme at atmospheric pressure. It is important to stress that this is the first report on biocatalysis at moderate pressures regarding immobilized GOX2 for bioconversion of D-glucose into D-glucosone by using compressed air.

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