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Bioconversion of D-glucose into D-glucosone by Glucose 2-oxidase from *Coriolus versicolor* at Moderate Pressures

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Abstract Glucose 2-oxidase (pyranose oxidase, pyranose:oxygen-2-oxidoreductase, EC 1.1.3.10) from *Coriolus versicolor* catalyses the oxidation of D-glucose at carbon 2 in the presence of molecular O_2 producing D-glucosone (2-keto-glucose and D-arabino-2-hexosulose) and H_2O_2. It was used to convert D-glucose into D-glucosone at moderate pressures (i.e. 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, [enzyme], [glucose], pH, temperature, nature of fluid and the presence of catalase. Glucose 2-oxidase was purified by immobilized metal affinity chromatography on epoxy-activated Sepharose 6B-IDA-Cu(II) column at pH 6.0. The rate of bioconversion of D-glucose increased with the pressure since an increase in the pressure with compressed air resulted in higher rates of conversion. On the other hand, the presence of catalase increased the rate of reaction which strongly suggests that H_2O_2 acted as inhibitor for this reaction. The rate of bioconversion of D-glucose by glucose 2-oxidase in the presence of either nitrogen or supercritical CO_2 at 110 bar was very low compared with the use of compressed air at the same pressure. The optimum temperature (55°C) and pH (5.0) of D-glucose bioconversion as well as kinetic parameters for this enzyme were determined under moderate pressure. The activation energy (E_a) was 32.08 kJmol^{-1} and kinetic parameters (V_{max}, K_m, K_{cat} and K_{cat}/K_m) for this bioconversion were 8.8 Umg^{-1} protein, 2.95 mM, 30.81 s^{-1} and 10,444.06 s^{-1}M^{-1}, respectively. The biomass of *C. versicolor* as well as the cell-free extract containing glucose 2-oxidase activity were also useful for bioconversion of D-glucose at moderate pressures. The enzyme was apparently stable at moderate pressures since such pressures did not affect significantly the enzyme activity.

Keywords Biocatalysis · Moderate pressures · Glucose 2-oxidase · *Coriolus versicolor* · Bioconversion of D-glucose into D-glucosone · Catalase · Kinetic parameters

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Introduction

Glucose 2-oxidase (pyranose oxidase, pyranose:oxygen-2-oxidoreductase, EC 1.1.3.10) is synthesized by several white rot fungi such as *Coriolus versicolor* and *Phanerochaete chrysosporium* and plays an important role in lignin biodegradation [1–5]. Traditional carbohydrate chemistry requires multiple and complex steps of activation and protection chemistry but it lack stereo-specificity in such reactions. Therefore, enzymes are ideal biocatalysts for stereo- and regioselective reactions which take place in aqueous medium under mild conditions [6]. Fungal glucose 2-oxidase 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 [7]. 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 [8]. As far as their chemical uses are concerned, dicarbonyl sugars represent interesting synthons since they combine the high number of functional groups and the inherent chiral properties of sugars with a high degree of chemical diversity in functional groups [6]. 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 glucose 2-oxidase and catalase into their corresponding keto sugars is very slow and time-consuming at atmospheric pressure (1 bar) and at room temperature (23°C) [9, 10]. Moreover, glucose 2-oxidase activity is highly unstable [11, 12], 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 [13]. The relative affinity of this enzyme for other carbohydrates is low at atmospheric pressure (1 bar) and at 23°C, which could be potentially increased by medium engineering at moderate to high pressures [14].

Enzyme reactions can be carried out under high pressure or in supercritical fluids which is a new and promising field of enzyme engineering [15]. High pressure is responsible for direct conformational changes in enzymes which affect their biological activities [16–18]. However, such high pressures may inactivate some enzymes since undesirable changes may occur in enzyme conformation which result in enzyme inactivation [17]. Moreover, pressure also influences the reaction rate constant which changes according to transition state theory and standard thermodynamics [19]. There are many reports in the literature on the effect of high pressure (i.e. up to 1 kbar) on enzyme structure and activity [16, 20]. However, few published work has 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 glucose 2-oxidase. Several parameters affecting this bioconversion will be investigated such as pressure, enzyme and D-glucose concentrations, pH, temperature and the presence of catalase.

Material and Methods

Materials

C. versicolor was isolated from old growth forest of Olympic Peninsula (Port Townsend, WA, USA). Corn-steep liquor was kindly donated by COPAM (Portugal); *o*-dianisidine,

iminodiacetic acid, 1,4 butanediol diglycydil ether, catalase and peroxidase were purchased from Sigma Chemical Company. Recombinant glucose 2-oxidase from *E. coli* was obtained from Biozyme Laboratories (UK). Sepharose CL-4B was obtained from GE Health Care (Sweden). DE-52 and Sugar Pak column was purchased from Whatman (UK) and Waters (USA), respectively.

Methods

Enzyme Production and Purification

Glucose 2-oxidase 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%), corn steep liquor (0.7%), $MgSO_4$ (0.15%) $CaCl_2$ (0.0125%) and KH_2PO_4 (0.1%) dissolved in tap water and adjusted the pH to 5.5 which was 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.

Erlenmeyer flasks (500 ml) containing 100 ml of sterile medium were inoculated with two pieces of 0.5×0.5 cm plugs of the appropriate culture grown in Petri dishes. The culture was grown at 200 rpm, 25°C for several days. By this time, it was possible to detect glucose 2-oxidase 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 glucose 2-oxidase 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 re-suspended in 2 volumes of 50 mM phosphate buffer pH 6.5 and sonicated at 100 W for 2 min in an iced bath. The suspension was centrifuged at 10,000×*g* for 5 min and the cell-free supernatant was the source of glucose 2-oxidase activity. The enzyme was purified by immobilized metal affinity chromatography on epoxy-activated Sepharose 6B-IDA-Cu(II) as described previously [21].

Enzyme Assay

Glucose 2-oxidase activity was determined by using *o*-dianisidine, D-glucose (0.1 M) and peroxidase (1 U) in 50 mM phosphate buffer pH 6.5 at atmospheric pressure (1 bar) and at 23°C. 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 cell-free extract. After 10 min, 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 (1 bar) and at 23°C by using 0.1 ml of appropriate concentration of D-glucose in 50 mM phosphate buffer pH 6.0 and suitable amount of either cell-free extract or purified enzyme in a final volume of 1 ml by bubbling either with pure oxygen or air. Aliquots were removed from the reaction mixture at suitable time intervals which were analysed by high-performance liquid chromatography (HPLC) in terms of substrate and product as described below.

Protein Assay

Protein concentration was determined by the Coomassie blue dye binding method [22].

Biocatalysis at Moderate Pressure

Apparatus set-up The experimental set-up, as shown in Fig. 1, consists basically of one batch reactor (Micro Reactor Parr Instruments, CO, 4843) with 25 ml capacity equipped with agitation, temperature and pressure reading devices. Two principal valves' inlet and outlet connections and a rupture disk set at 250 bar were used to perform the enzyme reactions.

When compressed air was used, the gas cylinder was connected directly to the reactor (as shown in Fig. 1). In each experiment samples were withdrawn at appropriate intervals to determine substrate and product concentration by using an HPLC system (Jasco Instruments).

Biocatalysis at moderate pressure was carried out by using either cell-free extract from *C. versicolor* (0.21 Umg^{-1} protein), purified commercial enzyme (8.2 Umg^{-1} protein), purified enzyme from *C. versicolor* (6.0 Umg^{-1} protein) or biomass from *C. versicolor* (0.1 g, wet weight). The reaction mixture contained appropriate concentration of D-glucose in 50 mM phosphate buffer pH 6.5 (19.9 ml) and 100 μl of appropriate form of enzyme which was stirred at 110 rpm (Fig. 1). The reaction was performed at several working pressures at 23°C by using compressed air and, in some experiments, catalase (20 mg, 1,000 Umg^{-1} protein) was added to the reaction mixture. 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.

HPLC Analysis and 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 5.17 bar, 90°C, 0.1 mM Ca(II) EDTA in Millipore water as the solvent and at a flow rate of 0.5 $ml\,min^{-1}$.

Samples (25 μl) from enzymatic 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 was 9.3 and 10.3 min, respectively. The peak area was determined as a function of either D-glucose or D-glucosone concentration.

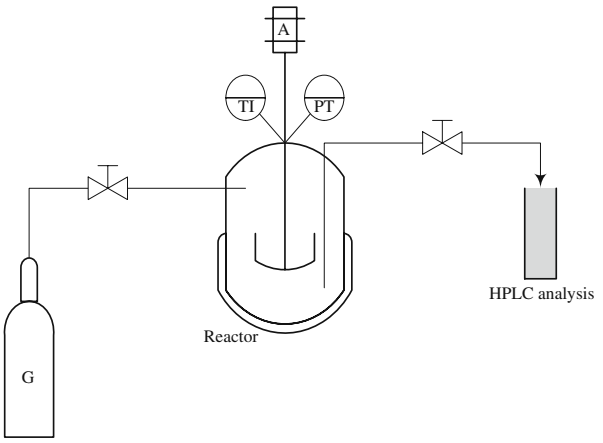

Fig. 1 Schematic diagram of the experimental apparatus—enzyme reactor unit. *G* gas cylinder, *A* magnetic stirrer device, *TI* and *PI* temperature and pressure indicators

Kinetic Characterization of Purified Preparation of Glucose 2-oxidase from C. versicolor at Moderate Pressures

Glucose 2-oxidase activity was assayed by using D-glucose as the substrate and one unit of glucose 2-oxidase activity was defined as the amount of enzyme required to oxidize 1 μmol substrate/min at 23°C and 50 mM phosphate buffer pH 6.5. Assays in pressure batch reactor were performed at suitable moderate pressures 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 glucose 2-oxidase activity was investigated at 100 bar by using purified enzyme preparation and buffer solutions were prepared containing 0.1 M D-glucose in several buffers as follows: 50 mM citrate buffer pH 3.0 to 6.0, 50 mM phosphate buffer pH 6.0 to 8.0 and 50 mM glycine buffer at pH 9.0 and 10.0. This mixture (19.9 ml) was used in batch reactor for biocatalysis in the presence of purified enzyme (0.1 ml). Suitable aliquots were removed from the pressure reactor at appropriate time intervals and analysed by HPLC as described above at 23°C. The optimum temperature of glucose 2-oxidase activity was investigated by using purified enzyme preparation 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 and 66°C) for 5 min. Suitable aliquots were removed from the pressure reactor at appropriate time intervals and analysed by HPLC as described above.

The activation energy for glucose 2-oxidase reaction was determined by measuring the enzyme activity at different temperatures and transforming them according to the Arrhenius equation:

$$\ln(v) = A - E_a/R \times 1/T$$

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

The kinetic constants (V_{max}, K_m, K_{cat} and K_{cat}/K_m) for D-glucose were determined by Michaelis–Menten plot.

Biocatalysis at Atmospheric Pressure (1 bar) and at 23°C

For comparison purposes, biocatalysis at 1 bar and at 23°C was also carried out by using either cell-free extract from *C. versicolor* (0.21 Umg^{-1} protein), biomass of *C. versicolor* (0.1 g wet weight), purified commercial enzyme (8.2 Umg^{-1} protein) and purified enzyme from *C. versicolor* (6.0 Umg^{-1} protein). The reaction mixture contained appropriate concentration of D-glucose in 50 mM phosphate buffer pH 6.5 (19.9 ml) and 100 μl of appropriate form of enzyme/biocatalyst which was stirred at 110 rpm in a magnetic stirrer. The reaction was performed by using the reaction mixture equilibrated with plain atmosphere ($[O_2]$=8.89 mg l^{-1}), the reaction mixture bubbled with air ($[O_2]$=8.89 mg l^{-1}) and the reaction mixture bubbled with pure O_2 ($[O_2]$=42.29 mgl^{-1}) at 23°C. The solubility of O_2 in the solution is assumed as in fresh water, using the toolbox and results from Engineering Toolbox [23] and the respective corrections when O_2 is pure against the concentration in the air.

In some experiments, catalase (20 mg, 1,000 Umg^{-1} protein) was added to the reaction mixture. Aliquots (100 μl) were removed from the reaction mixtures at suitable time intervals and processed the same way by HPLC as described earlier.

Storage Stability of Glucose 2-oxidase at Moderate Pressures

Purified glucose 2-oxidase (2.5 U) was incubated in 20 ml, 50 mM phosphate buffer pH 6.0 in batch reactor at 70 bar, 100 rpm at 23°C for several days. Aliquots were removed at suitable time intervals and glucose 2-oxidase activity was measured by HPLC as described above. For comparative purposes, the stability of the enzyme was also investigated at atmospheric pressure (1 bar) and at 23°C by using the same methodology as mentioned above.

Results and Discussion

Biocatalysis at Moderate Pressures and Atmospheric Pressure Conditions

The calibration curves for D-glucose and D-glucosone were carried out by HPLC which exhibited linear relationship between the peak area (mV) and either D-glucose or D-glucosone concentration (Fig. 2).

The formation of D-glucosone and the consumption of D-glucose were followed by HPLC when the reaction was carried out either at 1 bar and at 23°C or in batch reactor at 70 bar in the presence of the purified enzyme (Fig. 3). These data revealed that the enzyme reaction is much faster in a batch reactor (i.e 70 bar, $[O_2]$=2,960.0 mgl^{-1})) (Fig. 3a) than at atmospheric pressure (1 bar) (Fig. 3b). 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 (1 bar) conditions ($[O_2]$=8.89 mgl^{-1}). However, the activating effect of moderate pressures on glucose 2-oxidase activity cannot be ruled out since several reports in the literature have described this effect on some enzyme reactions [16, 24]. The effect of enzyme concentration on the initial velocity of this reaction was also investigated at moderate pressure of 100 bar which revealed that there is a linear relationship as shown in Fig. 4.

The oxidation of D-glucose in the presence of glucose 2-oxidase was also carried out at several working pressures at room temperature (23°C) as shown in Fig. 5a. These data suggest that by increasing the pressure there is an increase in the conversion of D-glucose compared with atmospheric pressure (1 bar) conditions because the concentration of O_2 increases with the pressure. Several experiments were carried out by increasing the pressure

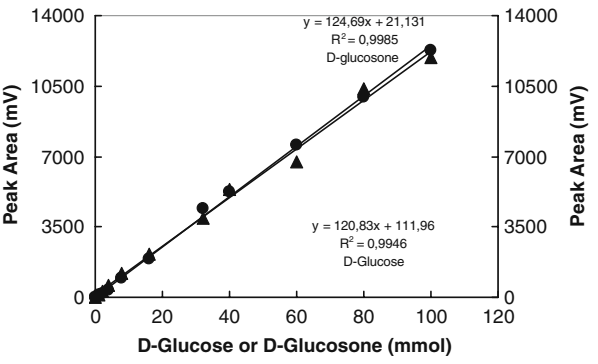

Fig. 2 Calibration curves for D-glucose (*filled triangles*) and D-glucosone (*filled circles*) by HPLC analysis

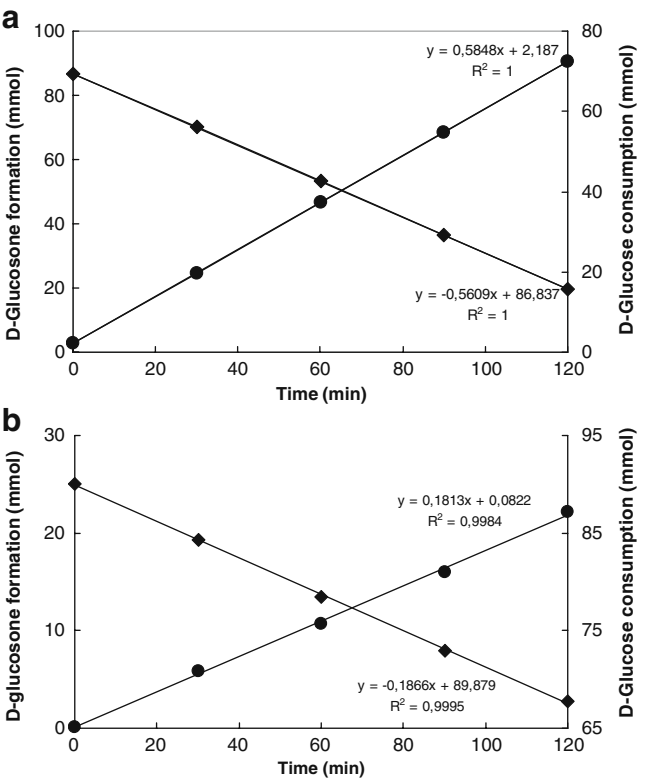

Fig. 3 Progress curve showing the consumption of D-glucose (*filled squares*) and formation of D-glucosone (*filled circles*) at 70 bar in the batch reactor (**a**) and at 1 bar and at 23°C (**b**) by using purified enzyme preparation (0.1 ml, 0.2 U, specific activity, 6.0 U/mg protein) from *Coriolus versicolor* without catalase

up to 160 bar in the batch reactor which exhibited an increase in rate of reaction (data not shown). The bioconversion of D-glucose was also performed at different [O_2] as shown in Fig. 5b which suggests that the highest bioconversion rate occurred in the pressure reactor followed by pure O_2 and air. Furthermore, the conversion of D-glucose into D-glucosone was investigated by using different enzyme preparations such as cell-free extract, biomass from *C. versicolor*, commercial enzyme and purified enzyme from *C. versicolor*. The data presented in Fig. 5c revealed that cell-free extract exhibited a higher degree of conversion

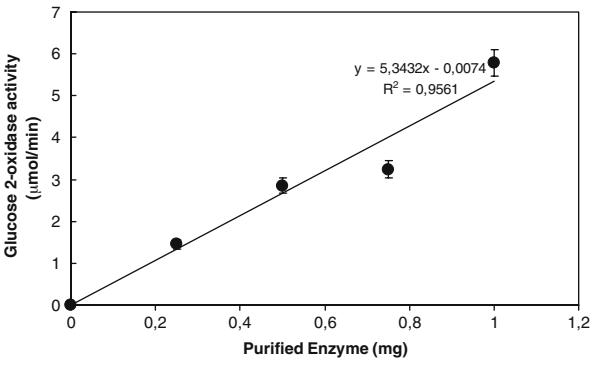

Fig. 4 Effect of purified enzyme concentration from *C. versicolor* on initial velocity of the reaction catalysed by glucose 2-oxidase. The bioconversion was carried out by using 100 mM D-glucose at pH 6.0, 100 bar, 100 rpm and increasing concentration of purified enzyme (6.0 U/mg protein) without catalase as described in Materials and methods

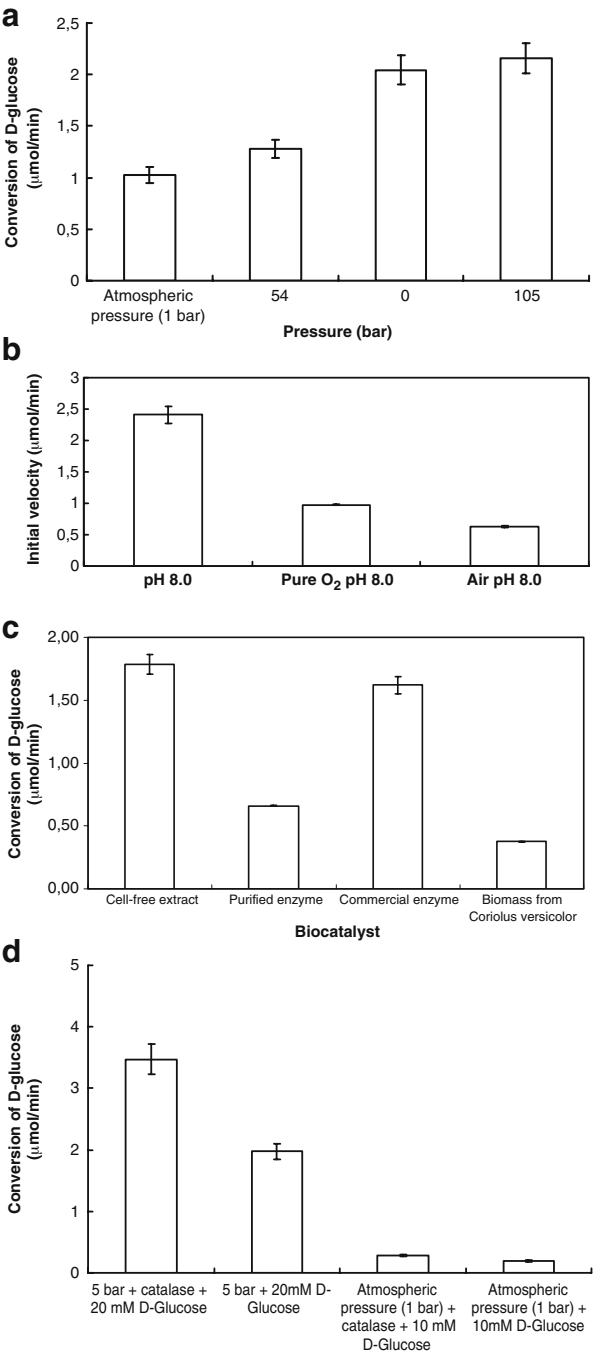

Fig. 5 **a** Effect of pressure on bioconversion of D-glucose into D-glucosone. The conversion of 10 mM D-glucose to D-glucosone was carried out in a batch reactor at 100 rpm for a period of 90 min at 23°C by using 100 μl (0.5 U) of purified enzyme (6.0 U/mg protein) without catalase. Enzyme assays were carried out by HPLC as described in Materials and methods. **b** Effect of [O_2] on bioconversion of D-glucose into D-glucosone. Conversion of 10 mM D-glucose to D-glucosone was carried out for a period of 90 min in the batch reactor at 100 bar and 100 rpm, by bubbling pure O_2 at room temperature and by bubbling air in the reaction mixture in 50 mM Tris buffer pH 8.0 by using 100 μl (1.0 U) of purified enzyme (6.0 U/mg protein) without catalase. Enzyme assays were carried out by HPLC as described in Materials and methods. **c** Effect of different biocatalysts on bioconversion of D-glucose into D-glucosone. This bioconversion was carried out for a period of 90 min in the batch reactor at 100 bar and 100 rpm by using cell-free extract (0.25 U, 0.21 U/mg protein), purified enzyme from *C. versicolor* (0.25 U, 6.0 U/mg protein), purified commercial enzyme (0.25 U, 8.2 U/mg protein) and biomass from *C. versicolor* containing glucose 2-oxidase activity (0.25 U) at 23°C without catalase. **d** Effect of catalase on bioconversion of D-glucose to D-glucosone in the presence of glucose 2-oxidase (0.5 U), which was determined at 1 bar and at 23°C and 5 bar and 100 rpm at 23°C for a period of 60 min of reaction

compared with the purified enzyme. Surprisingly, the biomass of *C. versicolor* containing glucose 2-oxidase 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 cell-free extract may be due to mass transfer limitations (Fig. 5c), which has been reported by several researchers [25, 26].

The presence of catalase in the reaction mixture was also investigated on the degree of conversion as shown in Fig. 5d. These data revealed that the presence of catalase increased significantly the degree of conversion suggesting that hydrogen peroxide acts as a powerful inhibitor for this enzyme, which is in agreement with published reports [11].

Kinetic Characterization of Purified Preparation of Glucose 2-oxidase from *C. versicolor* at Moderate Pressures

The bioconversion of D-glucose in the presence of glucose 2-oxidase was carried out at several working temperatures as shown in Fig. 6a. These data suggest that optimum temperature for maximum conversion of D-glucose into D-glucosone under these experimental conditions is 55°C which is higher than the value of 50°C reported for this enzyme at 1 bar [27]. From the linear range of the Arrhenius plot, the activation energy (E_a) was determined to be 32.08 kJmol^{-1}, which is in agreement with the data published for this enzyme at 1 bar [9, 28]. The effect of pH on the bioconversion of D-glucose into D-glucosone at high pressure was also investigated which revealed that the optimum pH is 5.0 for maximum conversion of D-glucose into D-glucosone by using cell-free extract containing glucose 2-oxidase activity (Fig. 6b). The same optimum pH for bioconversion of D-glucose was found when purified enzyme preparation was used (data not shown). These data are markedly different from the value of 6.2 reported for this enzyme at atmospheric pressure (1 bar) and at 23°C conditions [8, 27].

The effect of D-glucose on initial velocity of glucose 2-oxidase-catalysed reaction at moderate pressures (i.e. 100 bar, [O_2]=4,228.7 mgl^{-1}) was carried out by using a purified enzyme preparation (Fig. 7). The kinetic parameters (i.e. V_{max}, K_m, K_{cat} and K_{cat}/K_m) were determined by using Sigma Plot, and were found to be 8.8 Umg^{-1} protein, 2.95 mM, 30.81 s^{-1} and 10,444.06 $s^{-1}M^{-1}$, respectively. These results are slightly different from the data reported in the literature which may be due to different assay conditions for this enzyme since linked enzyme assays with peroxidase were used in such kinetic studies [27, 28].

This bioconversion of D-glucose was also studied by using either supercritical CO_2 at 35°C or compressed nitrogen at 110 bar (data not shown). The results obtained suggest that the degree of conversion is very low when either supercritical CO_2 or N_2 were used compared with the compressed air since the oxidation of D-glucose catalysed by glucose 2-oxidase requires the presence of O_2 as the substrate which is absent in either supercritical CO_2 or N_2 (data not shown).

Storage Stability of Glucose 2-oxidase at Moderate Pressures

The stability of glucose 2-oxidase activity at moderate pressures was investigated which revealed that it did not affect significantly the enzyme activity at 70 bar compared with the atmospheric pressure (1 bar) and at 23°C conditions as shown in Fig. 8. In fact, the storage stability of the enzyme in these conditions, at moderate pressures and atmospheric pressure conditions, was investigated for 3 days, and results showed that there was no significant change in enzyme activity (Fig. 8).

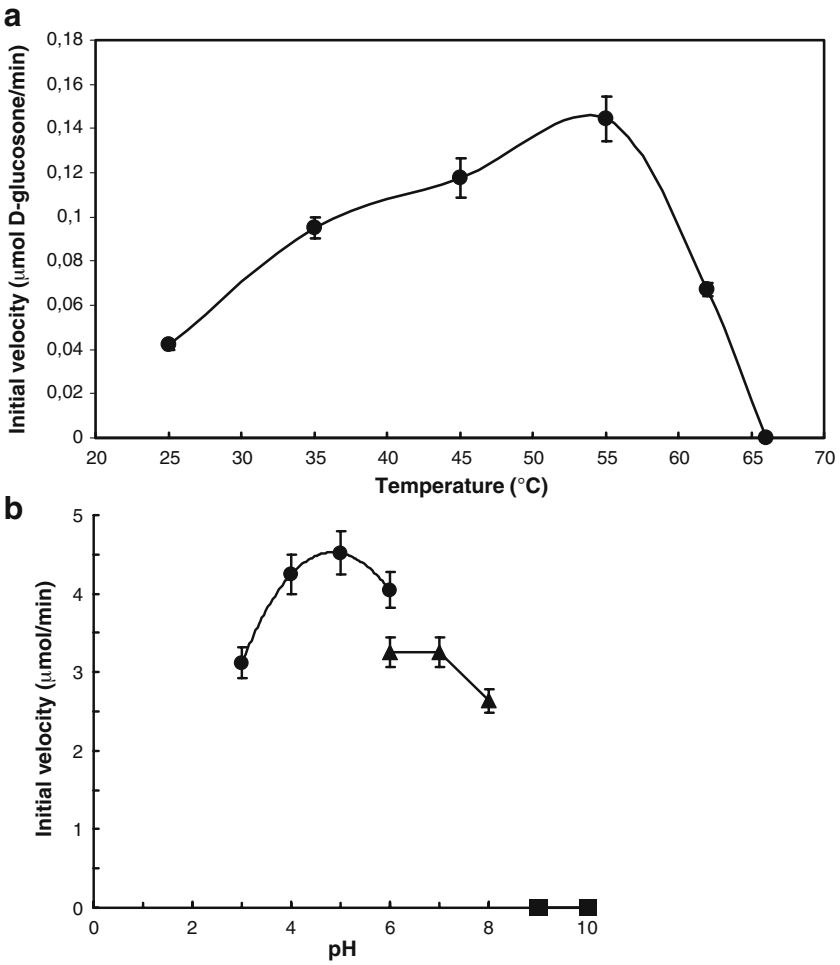

Fig. 6 **a** Effect of temperature on bioconversion of D-glucose to D-glucosone by using purified enzyme (0.1 U). The reaction was carried out at 100 bar, 100 rpm and 100 mM glucose at pH 6.5 by using purified enzyme (6.0 U/mg protein) without catalase. **b** Effect of pH on bioconversion of D-glucose to D-glucosone by using purified enzyme. The reaction was carried out at 100 bar, 100 rpm and 100 mM glucose at different pH values by using purified enzyme (0.8 U, 6.0 U/mg protein) without catalase. 50 mM citrate buffer pH 3.0 to 6.0 (*filled circles*), 50 mM phosphate buffer pH 6.0–8.0 (*filled triangles*) and 50 mM glycine buffer at pH 8.0 and 9.0 (*filled squares*)

To our knowledge, this is the first report on biocatalysis at moderate pressures regarding glucose 2-oxidase for bioconversion of D-glucose into D-glucosone by using compressed air. On the other hand, this is also the first report on the use of either cell-free extract or biomass for bioconversion of D-glucose at moderate pressures.

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: temperature,

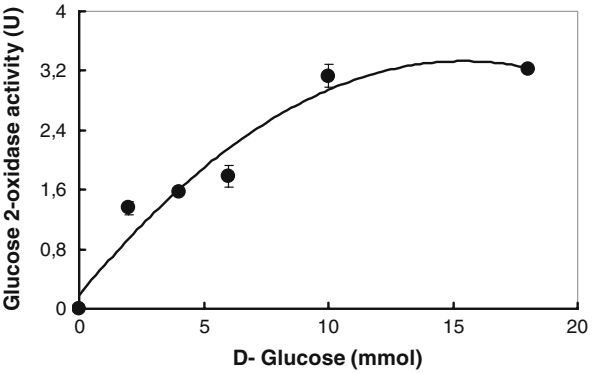

Fig. 7 Effect of D-glucose concentration on the initial velocity of the reaction catalysed by glucose 2-oxidase. D-Glucose concentration was varied in the reaction mixture containing purified enzyme (1 U, 6.0 U/mg protein) in 50 mM phosphate buffer at pH 6.0, 100 rpm and 100 bar at 23°C

pH, enzyme concentration, glucose concentration and the presence of catalase. The degree of bioconversion of D-glucose was found to be higher at moderate pressures than at atmospheric pressure (1 bar), which could be explained due to high [O_2] at moderate pressure and O_2 is one of the substrates for this enzyme reaction. However, the potential activating effect of moderate pressure on enzyme activity cannot be neglected since several enzyme reactions are activated by moderate pressures [16, 24]. On the other hand, the purification of glucose 2-oxidase from *C. versicolor* is not required since either the cell-free extract or the biomass from *C. versicolor* were found useful for conversion of D-glucose into D-glucosone. The optimum pH and temperature for this reaction at moderate pressures were different compared with the data published for this enzyme at 1 bar [8, 27]. It is important to stress that this is the first report on biocatalysis at moderate pressures regarding glucose 2-oxidase for bioconversion of D-glucose into D-glucosone by using compressed air.

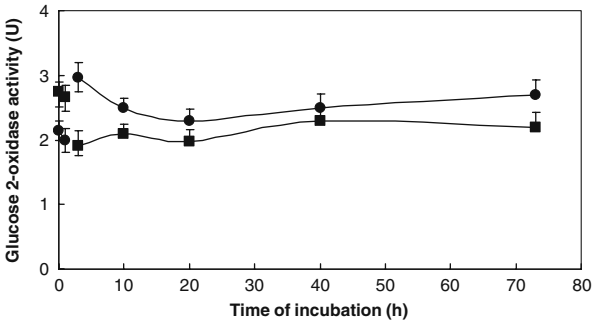

Fig. 8 Storage stability of glucose 2-oxidase at moderate pressures. Purified glucose 2-oxidase (2.5 U) was incubated in 20 ml 50 mM phosphate buffer pH 6.0 in batch reactor at 70 bar (*filled circles*), 100 rpm at 23°C for several days. Aliquots were removed at suitable time intervals and glucose 2-oxidase activity was measured by HPLC as described in Materials and methods. For comparative purposes, the storage stability of the enzyme was also investigated at 1 bar and at 23°C (*filled squares*) conditions by using the same methodology as mentioned above

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