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Investigation of biocatalytic properties of soybean seed hull peroxidase

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Abstract

Soybean seed hull peroxidase (SBP) is an inexpensive oxidoreductive enzyme and could potentially be used to oxidise/polymerise various organic pollutants present in the industrial and petrochemical wastes. SBP is able to retain its catalytic properties under wide ranges of pH and at elevated temperatures. In this study, a systematic evaluation of the biocatalytic properties of SBP was carried out. The optimal pH for SBP activity is pH 6.0 and significant activity was observed between 2.2 and 8.0. SBP also showed three times higher activity at an elevated temperature of 80°C and at pH 6.0 when compared to the activity at room temperature. The pH and temperature of the reaction mixture were found to significantly influence the SBP activity. SBP is fairly active in organic solvents. The enzyme exhibited highest activity in the presence of 16.67% (w/v) ethanol followed by acetone, methanol and acetonitrile. The enzyme activity was reduced with an increase in concentration of the organic solvent. SBP also showed maximum activity at different concentrations of acetone using a phosphate buffer, pH 6.0 than with the other pH buffers. Benzene/acetone mixture seems to be another better solvent system for SBP where it showed about 65% of its activity at 16.67% (w/v) concentration.

Keywords: Soybean seed hull peroxidase; Catalytic properties; Buffer; Organic solvents

1. Introduction

Plant peroxidases are oxidoreductive enzymes, possess a wide variety of substrate specificity and can oxidise a large number of aromatic compounds in the presence of H_2O_2 [1,2]. Because of this property, plant peroxidases have been extensively used for the catalytic oxidation of phenolic and other aromatic contaminants in industrial wastewater by H_2O_2 to form aromatic-free radicals that subsequently continue to form high molecular weight products, which precipitate from solution due to their low solubility. This method envisages a possible alternative for the industrial wastewater treatment when conventional methods, such as biological treatment, activated carbon and advanced oxidation may be ineffective due to the nature of the contaminants in the wastewaters [3–5]. Extensive research was previously carried out on the use and characterisation of horsh radish peroxidase among plant peroxidases. The prohibitive cost of commercial HRP led to the search for alternate cheaper sources of other plant peroxidases, to substitute HRP in various applications [6].

Soybean peroxidase (SBP), one among the other plant peroxidases, is an inexpensive by-product of

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soybean seed hulls. It can be detected in root, leaf and seed hulls of the soybean. The peroxidase obtained from seed hulls contains the highest activity compared to the peroxidase obtained from the other parts of soybean plant [7]. SBP's marketed uses range from medical diagnostic tests to removal of chlorine-containing pollutants from industrial wastewater. An enzyme from soybean hulls is now replacing formaldehyde in adhesives, abrasives, protective coatings, and other products [8]. However, very few reports are available on the biocatalytic characterisation of SBP. In view of SBP's inexpensive nature and potential applications, much attention has to be paid on its biocatalytic efficacy in aqueous as well as in organic solvents because of the nature of pollutants in the industrial wastewater. Use of organic solvents for enzymatic reactions has several advantages, which include increased solubility of organic substrates and shifting the hydrodynamic equilibria to favour synthesis over hydrolysis [9]. In this paper, we report on the biocatalytic properties of SBP in aqueous and organic solvents.

2. Material and methods

Soybean seed hulls were obtained from ADM Agro-Industries, Windsor, Ont., Canada. All chemicals used in this study were of analytical grade and obtained from commercial sources. Crude SBP extract was prepared as follows. A weighed amount of (125 g) soybean hulls were soaked in 1000 ml phosphate buffer (10 mM NaH_2PO_4/NaOH, pH 6.0) at 4°C for 24 h. The mixture was then centrifuged at 3000 rpm for 15 min. The supernatant was filtered using Whatman No. 4 filter paper. The filtrate was then subjected to a second centrifugation at 13 000 rpm and 4°C for 10 min. The final supernatant was collected and stored at 4°C and used as source of crude SBP enzyme.

SBP is able to oxidise guaiacol and form an orange/red dye, tetra-guaiacol, in the presence of H_2O_2. The oxidised product has apparent absorbance in the range 400–500 nm. This property has been used to measure the activity of SBP [7,10]. In this study, the samples of crude SBP extract were diluted by 200–250 before measurement. The assay mixture contained 2000 μl diluted enzyme solution, 500 μl 1% (v/v) guaiacol, and 500 μl 0.3% (w/v) H_2O_2. The linear absorbance change of the assay mixture was measured at 420 nm using enzyme kinetics program on a UV–visible spectrophotometer (Cary 50 Bio, Varian, Australia).

For the measurements of pH effect on SBP activity, a series of buffer solutions were formulated according to Perrin and Dempsey [11] (pH 2.2–3.5, citric acid/NaOH buffer; pH 4.0–5.0, sodium acetate/acetic acid buffer; pH 6.0–8.0, Na_2HPO_4/NaH_2PO_4 buffer; pH 9.0–10.0, boric acid, KCl/NaOH buffer). The samples of crude SBP extract were diluted 200-fold using those buffers and their activities were then measured in accordance with the above-described method. For the experiments of temperature effect on SBP activity, the glass cuvette containing 2000 μl diluted enzyme solution and 500 μl 1% (v/v) guaiacol was heated by water bath to a pre-set temperature. Immediately, 500 μl 0.3% (w/v) H_2O_2 was added to the cuvette and the initial change in absorbance per minute at 420 nm was measured (one unit (U) of specific SBP activity (U) $= \Delta A_{420}\,\mathrm{min}^{-1}\,\mu\mathrm{g}^{-1}$ total protein).

For the experiments involving organic solvents, the same enzyme protocol described above was followed, except the enzyme was pre-diluted with different ratio of water and organic solvent mixture in the range 15–70% (w/v). For experiments involving non-miscible solvent, benzene, at first it was dispersed as fine droplets in water phase at high agitation rate (600 rpm) and then the enzyme activity was determined as described above.

The shelf life of SBP extracted from soybean seed hulls was monitored at room temperature and at refrigeration temperature (4°C). The crude SBP in solution form is stored at room temperature lost 35% of its activity within first 3 days. But when the crude enzyme is stored at 4°C, it was stable for two and half months and retained 99% of its activity.

3. Results and discussion

The major biocatalytic properties measured, which influence the activity of SBP were pH and temperature of the reaction mixture, and the nature of organic solvent. First, the effect of pH is discussed followed by temperature and effect of solvent.

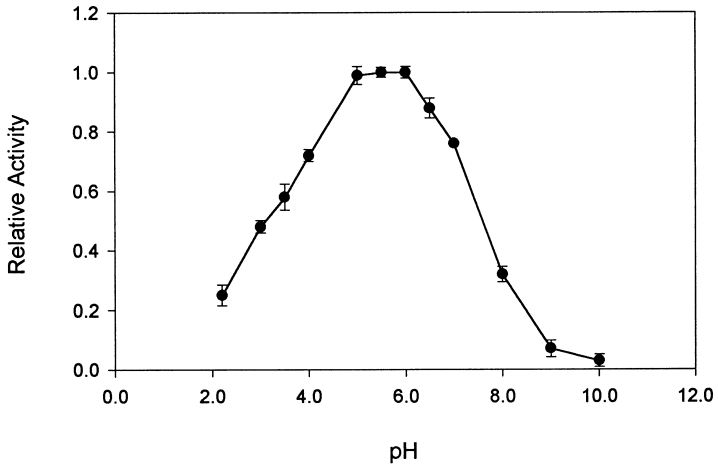

Fig. 1. Relative activity of SBP under different pH buffers at 25°C.

3.1. Effect of pH

The relative SBP activities as a function of pH are presented in Fig. 1. Relative activity is expressed as the rate of tetra-guaiacol formation at a particular pH normalised with respect to the highest rate. Experiments were performed using different buffer solutions to maintain the identical pH conditions in the pH ranging from 2 to 10. Fig. 1 indicates that SBP was active over a wide range of pH with a maximum SBP activity between pH 5.5 and 6.0. More than 95% of enzyme activity was observed at pH 5.0, about 90% at pH 6.5, and >50% between pH 3.0 and 7.0. About 25 and 32% enzyme activity was observed at pH 2.2 and 8, respectively. A similar trend was reported with little shift in pH for SBP activity in aqueous phenol by Nicell and Wright [3]. These results indicate that SBP from seed hulls retain significant activity over a wide range of pH conditions and thus demonstrates its ability to oxidise different substrates having broader range of pH.

3.2. Combined effect of pH and temperature

The combined effect of pH and temperature on SBP activity was studied by varying the reaction temperature between 20 and 95°C. Initially, the pH of the reaction mixture was maintained at 6.0 as it resulted in highest enzymatic activity (Fig. 1). Fig. 2 demonstrates the temperature profiles of SBP activity at different pH. Interestingly, at pH 6.0, the highest enzymatic activity was observed at the temperature of 80°C and is about three times higher than the activity obtained at room temperature. More than 95% of the highest enzyme activity was retained between 75 and 95°C, and >80% between 60 and 85°C. The rate of enzyme reaction is favoured at higher temperatures. Most of the organic pollutants are soluble in aqueous phase at higher temperatures. Hence increase in reaction temperature would result in increase in the rate of reaction of SBP against these substrates, as SBP is stable and active at elevated temperatures. From these observations, it can be postulated that SBP can be perceived to polymerise/oxidise most of the aromatic organic compounds presented in the industrial wastewater relatively at higher rate compared to many plant/microbial peroxidases.

Most of the organic pollutants (such as chlorophenol, etc.) are soluble at neutral or alkaline pH. Their solubility reduces in acidic pH. In order to find out the influence of temperature and reaction pH on SBP activity at neutral and towards alkaline pH side, experiments were conducted at pH 7.0 and 8.0, respectively, by incubating the reaction mixture at different temperatures ranging from 20 to 95°C. Highest SBP activity was observed at temperatures 75 and 80°C in both the cases of pH at 7.0 and 8.0, but the relative activity was just only 2 and 1.2 times of its original activity at room temperature, respectively (Fig. 2). The SBP activity was significantly lowered in case of pH at 8.0, when compared to the activity pH at 6.0 and 7.0. But

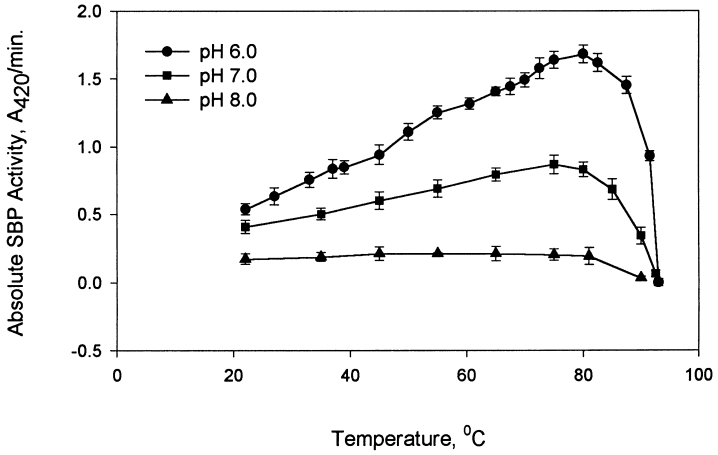

Fig. 2. Combined effect of temperature and pH on SBP activity.

at pH 8.0, the enzyme expressed wider stability and activity, more than 95% between the temperatures 37 and 80°C when compared to its activity at room temperature. These results indicate that the combined effect of temperature and reaction pH greatly influences the activity of SBP.

3.3. SBP activity in organic solvents

Enzymes ubiquitously exhibit their activity predominantly in aqueous phase. However, some enzymes are active in the presence of organic solvents with some proportion of aqueous phase. In order to find out whether or not the SBP is active in organic solvents, experiments were carried out using different organic solvents such as acetone, ethanol, methanol, and acetonitrile. The organic solvent that is being used as a medium affects the activity of the soybean enzyme. Some organic solvents seem to be better suited than others for use with enzymatic reactions. Fig. 3 shows the effect of different organic solvents on an average relative activity of SBP at pH 6.0. From these findings,

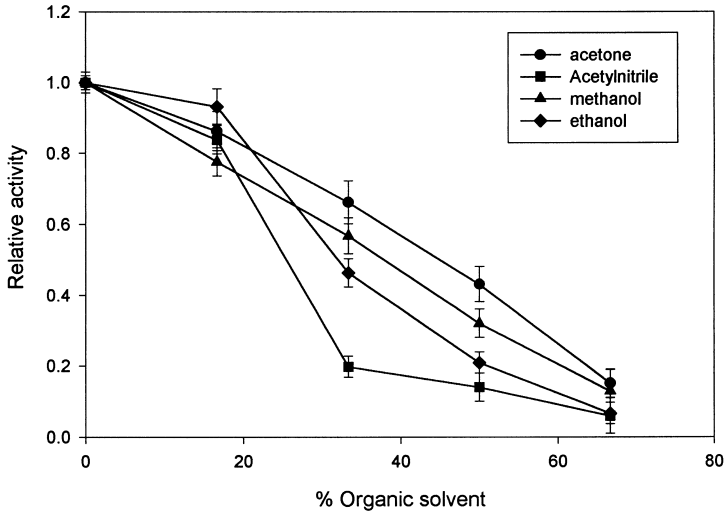

Fig. 3. Effect of different organic solvents on the relative SBP activity.

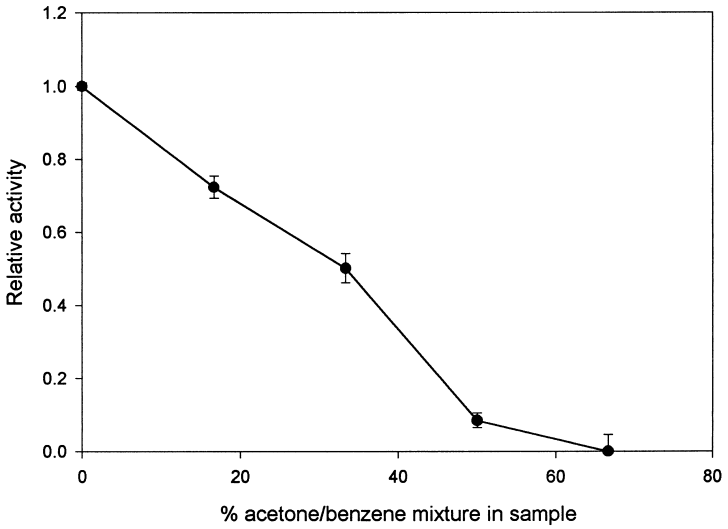

Fig. 4. Effect of benzene/acetone mixture on the relative activity of SBP.

it can be concluded that the enzyme exhibits maximum activity in acetone compared to the other organic solvents tested. The next best solvent is methanol, then ethanol and followed by acetonitrile.

The water to organic solvent ratio affects the activity of the enzyme. As the percentage of organic solvent in the sample increased, the relative activity of the enzyme decreased. This result was an expected one since the enzyme activity in low water conditions tends to be restricted and therefore reduced. The use of organic solvent is advantageous over aqueous phase alone, as it renders the enzyme to act against a variety of organic pollutants, which are highly soluble in organic solvent and hydro-organic mixtures.

The combination of benzene and acetone seems to also be a good organic solvent mixture system. The data for the benzene/acetone mixture using the SBP in the phosphate buffer, pH 6.3, is presented in Fig. 4. At 16.67% (w/v) of benzene/acetone mixture, about 65% of the normalised activity of the enzyme was observed. The advantage of using benzene is that it is one of the most common solvents used to dissolve many organic non-polar substrates. Also benzene is an excellent solvent for certain elements such as sulphur, phosphorous and iodine, for gums, fats, waxes and resins, and for most simple organic chemicals. Therefore, further experiments involving SBP applications and benzene solvent system can be possibly performed.

The effect of the pH of the reaction mixture on SBP activity in the presence of acetone was studied with different buffers ranging the pH from 2.25 to 12. Since acetone resulted in highest enzyme activity, it is chosen as a model solvent system for this study. Fig. 5 shows that the enzyme exhibits maximum activity using a buffer with a pH of 6.0. As observed earlier, the general trend seems to be that as the percentage of acetone increased, the normalised activity decreased. The maximum SBP activity is shown to be at a pH of 6.0, then 2.25, 8.15, 4, 12, and 10, respectively.

As with any other enzyme, the best medium for enzymatic activity for SBP is water. But depending on the applications and utilisation of SBP, a system made up of mostly organic solvents may be more beneficial, when observing enzyme activity, than a system containing mostly water. Different organic solvent systems affected the activity of SBP enzyme, but the enzyme was still found to be fairly active. The activity of the enzyme also depends on the organic solvent being used as a medium and on the ratio of water to organic solvent. As the amount of water in the system is reduced, the activity of the enzyme is also reduced. Using various types of buffers with different pH values to make SBP enzyme has also been shown to affect the activity of SBP enzyme.

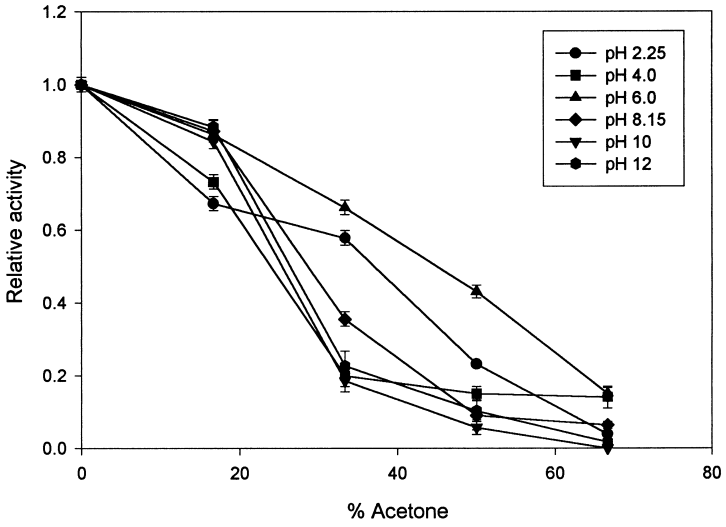

Fig. 5. Profiles of relative SBP activity in different concentrations of acetone in various pH buffers.

4. Conclusions

From the above findings, it is concluded that SBP extracted from soybean seed hulls is a highly robust enzyme and possesses greater stability and active under wide pH conditions and at elevated temperatures. Also this enzyme is fairly active in the presence of organic solvents such as acetone, acetonitrile, methanol, ethanol and benzene/acetone mixture, which widens the applicability of SBP for the treatment against a variety of organic pollutants present in the industrial and petroleum wastes.

Acknowledgements

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