Asian Journal of Chemistry; Vol. 25, No. 11 (2013), 5887-5890

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.12659

Removal of Bisphenol A by Soybean Hulls Peroxidase

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(*Received*: 21 May 2012; *Accepted*: 16 April 2013) AJC-13361

The soybean hulls peroxidase is known to remove chemical compounds from aqueous pollutant solutions. This study evaluates the potential of the soybean hulls peroxidase in the degradation of aqueous bisphenol A. Soybean hulls peroxidase (EC 1.11.1.7) was extracted and purified from soybean hulls. The molecular weight of the soybean hulls peroxidase is 37 kDa, determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Some physicochemical properties of the native enzyme, such as pH and temperature, were evaluated in order to determine the optimum conditions for the enzyme performance. We found the activity of soybean hulls peroxidase was higher at pH 5.0 and 50 ºC. For the removal of bisphenol A tested, the initial amount of H_2O_2 and the soybean hulls peroxidase were detected. The results indicated that the degradation of bisphenol A was over 80 % at soybean hulls 2 g/25 mL. This study verifies the viability of the use of the soybean hulls peroxidase in the biodegradation of the simulated bisphenol A pollution.

Key Words: Soybean hulls peroxidase, Bisphenol A, Removal.

INTRODUCTION

The threat of hormone disruptors, included polychlorinated biphenyl, polycyclic aromatic hydrocarbon, bisphenol A and other chemical pollutants, has emerged as a worldwide environmental concern. Bisphenol A is the popular raw material in the production of polycarbonate plastic. Bisphenol A, as a classic environmental hormones, are believed to disrupt the endocrine system in humans and animals[1]. And now, more countries proposed legislation to ban bisphenol A in baby bottles and food packages. To reduce the pollutant to the water environment and organisms, it is necessary to seek out the effective method to remove bisphenol A from water. The main techniques are applied in the area of wastewater treatment are physical adsorption, chemical oxidation degradation, biodegradation and others. In the biological treatment process, due to enzymes' advantages of efficiency and environment friendly, the enzyme are received much more attention and applied in effluent treatment.

Peroxidases (EC 1.11.1.x), as an oxido-reductase, have been known to catalyze the oxidation of the variety of organic and inorganic compounds form polymeric products through the production of free radicals in the presence of hydrogen peroxide[2]. During the past several decades, it has been conducted to investigate the new possibilities offered by plant peroxidases in this field. Most applications of plant peroxidases have focused on the treatment of phenols, chlorophenols and other aromatic organic contaminants in different types of effluents[3-6]. Plant peroxidase is very effective in removing pollutants, but it always requires the presence of H_2O_2. After the organic contaminants are oxidized to corresponding free-radical formation by H_2O_2, peroxidase can catalyze the chemical substrates to transform into less biodegradable polymeric compounds which could be easily removed by subsequent filtration and flocculation[4-6]. Plant peroxidases that have been used for the laboratory-scale treatment of aqueous aromatic contaminants include horseradish peroxidase (HRP, EC 1.11.1.7), soybean hulls peroxidase and a number of other peroxidases from different sources. More research results demonstrated that application of purified horseradish peroxidase to degrade certain recalcitrant organic compounds such as phenols and substituted phenols with high efficiencies from waste waters and drinking water[6-8].

Compared to horseradish peroxidase, there are more pollutants are not studied on the removal or treatment by soybean hulls peroxidase. However, more results indicated that the efficiency of soybean hulls peroxidase is equivalent to horseradish peroxidase, even higher[9], at the degradation of phenols or chlorophenols. The soybean hulls peroxidase always used in the removal of phenols and chlorophenols[9-18]. Bodalo *et al.*[18,19] indicated that free soybean hulls peroxidase almost able to complete (95.7 %) removal of 4-chlorophenol

with half enzyme concentration (10 mg/L) that necessary for free horseradish peroxidase (20 mg/L) which eliminated 88.1 % of the initial 4-chlorophenol content. On the other hand, soybean hulls peroxidase removed 5 % more of 4-chlorophenol than horseradish peroxidase at an immobilized enzyme. However, Bassi *et al*.[16] used soybean seed hulls directly in removal of pollutants. A satisfactory removal (80-96 %) of target phenol (10.6 mM) and chlorophenols (3.1-3.9 mM) was achieved. These results demonstrate that soybean seed hulls, compared to purified soybean hulls peroxidase, may be cost-effective alternative in the enzymatic removal of phenolic compounds through polymerization reactions. Soybean hulls peroxidase efficiently removed aromatic compounds from synthetic wastewater in the presence of H_2O_2.

Although, there are some peroxidase applied in the removal of chemical pollutants in the area of water treatment. There are little researches on the removal of environmental hormones in the presence of peroxidase and H_2O_2. This kind of contaminants would be as the object of soybean hulls peroxidase potential application in this study. Many treatments can be efficient in removal of pollutant, however, it is necessary to evaluate whether is effective in different conditions. In this study, the use of the enzyme soybean hulls peroxidase and soybean hulls in the removal of bisphenol A were discussed. Some parameters were studied to improve the removal efficiency.

EXPERIMENTAL

Soybeans (Glycine max) was purchased from local supermarket and stored at room temperature. Bisphenol A, guaiacol, H_2O_2 (30 %) were purchased from Sigma-Aldrich Fine Chemicals. Other agents and chemicals were used of analytical reagent grade and without further purification.

Extraction of soybean hulls peroxidase: In this study, soybean hulls peroxidase enzyme are isolated from soybean hulls as the reported procedure[19]. Firstly, the dry seeds were washed by distilled water and then soaked in distilled water for approximately 24 h or until the seed coats were easily removed. Secondly, this tissue was frozen in liquid N_2, ground to fine powder and extracted in buffer which is usually phosphate buffer (Na_2HPO_4/NaH_2PO_4 buffer solution, 0.1 mol/L, pH 6.0) at a ratio of probably 10 mL extraction buffer per 1 g tissue. The most important is that all purification steps must be performed at 4 ºC or on ice. Thirdly, the samples were centrifuged at 6000 rpm for 15 min, filtered through filter paper to remove the soybean hulls fragment and centrifuge again at 15000 rpm for 10 min. The deposition was re-dissolved in the phosphate buffer solution. The final supernatant was stored at 4 ºC and used for the characterization of crude soybean hulls peroxidase extract and followed experiments. The supernatant containing the soybean hulls peroxidase enzyme was used for further studies.

Soybean hulls peroxidase assay: Soybean hulls peroxidase is able to oxidize guaiacol to tetra-guaiacol and form a tea-brown dye, in the presence of H_2O_2. The oxidized product has apparent absorbance in the wavelength of 436 nm. This property has been used to measure the activity of soybean hulls peroxidase, which was measured using phosphate buffer of pH 6, H_2O_2 as substrate and guaiacol as chromogen. Then 1 mL of each enzyme sample was added to 3 mL of substrate (guaiacol and H_2O_2). After 1 min of incubation, the absorbance of the assay mixture was measured at 436 nm on an UV-visible spectrophotometer. In this experiment, soybean hulls peroxidase activity unit (U) is defined by the absorbance value increase 0.001 at optical density 436 nm per minute, at room temperature and pH 6 conditions.

In these studies, we use the soybean hulls peroxidase concentration to evaluate the soybean hulls peroxidase activity in the solution.

$$\text{Relative SBP activity} = (C_0\text{-}C_t)/C_0$$

where C_0 and C_t are the initial and final concentrations of soybean hulls peroxidase, respectively.

Bisphenol A detection: Weight 0.1 g bisphenol A powder and then dissolve it by 100 mL methanol, the concentration of initial bisphenol A stock solution is 1 mg mL^{-1}. It will be stored at 4 ºC and dark place for further use. Bisphenol A was analyzed using HPLC method with column (25 mm × 4.6 nm id, 5 µm particles) and UV detector (277 nm). The mobile phase consisted of 60:40 acetonrile/water at a flow rate of 1.0 mL min^{-1} [20,21]. Adjust the concentration of bisphenol A to 10, 20, 30, 40, 50 and 100 µg/mL and then detect the peak area by HPLC to make the bisphenol A concentration standard curve.

Removal of bisphenol A by soybean hulls peroxidase: The removal experiments of bisphenol A were carried out in 125-mL conical flasks, which were placed on a shaker with temperature and speed control. The whole reaction volume was 25 mL, adjust the soybean hulls peroxidase concentration after the whole removal experiment, the samples were centrifuged to remove the sediment at 6000 rpm for 10 min. Then, the bisphenol A concentrations were detected by HPLC. The percentage removal of bisphenol A was calculated using the following relationships:

$$\text{Bisphenol A removal rate (\%)} = (C_0 - C_t)/C_0 \times 100\ \%$$

where C_0 and C_t are the initial and final concentrations of bisphenol A, respectively.

RESULTS AND DISCUSSION

Characterization of soybean hulls peroxidase: The crude extraction of soybean hulls peroxidase was proved by SDS-PAGE. The objective of this experiment is to prove the extracted crude enzyme from soybean hulls contain major composition of soybean hulls peroxidase. The SDS-PAGE of soybean hulls peroxidase (Fig. 1) showed that the left lane is the standard protein molecular marker and the right lane is crude extraction of soybean hulls peroxidase. Compare the protein marker, except some different molecular weight of protein in the solution, the weight of 37 KDa of soybean hulls peroxidase is still in the extraction. In addition, combined with soybean hulls peroxidase enzymatic activity determination experiment, we can conclude that there are active soybean hulls peroxidase in the soybean hulls peroxidase extraction.

Effect of temperature on soybean hulls peroxidase activity: Most of the enzymes have major challenges associated with application of peroxidase is that poor thermal and environmental stability limits the large-scale applications of

peroxidase catalysis[22]. The most commonly studied plant peroxidase, for example horseradish peroxidase, is rapidly inactivated at temperatures above 65 ºC[23]. Compare to horseradish peroxidase and other peroxidase, soybean hulls peroxidase retains catalytic activity over wide ranges of temperature[24,25]. It has an unusually high thermal stability, being active at 70 ºC while most plant peroxidases are denatured[13]. The high thermostability of soybean hulls peroxidase may have advantages in a number of processes with demonstrated need for thermostable peroxidases in wastewater treatment[12]. Coupled with its high oxidation potential, soybean hulls peroxidase's high thermostability makes this peroxidase an intriguing catalyst for commercial and environmental applications[24].

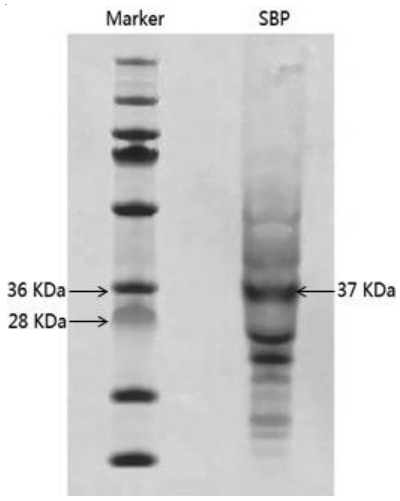

Fig. 1. SDS-PAGE of soyabean peroxidase and protein marker

Based on soybean hulls peroxidase's special properties in thermodynamic performance, the temperature for free soybean hulls peroxidase was chosen to be 30, 40, 50, 60 ºC and the enzyme activity at which maximum activity attained was determined using enzyme relative activities. Fig. 2 concluded that soybean hulls peroxidase has significantly high thermal quality and the optimum temperature range is about 50 ºC. It is concluded that soybean hulls peroxidase is stable at relatively higher temperature condition. This is consistent with other literatures.

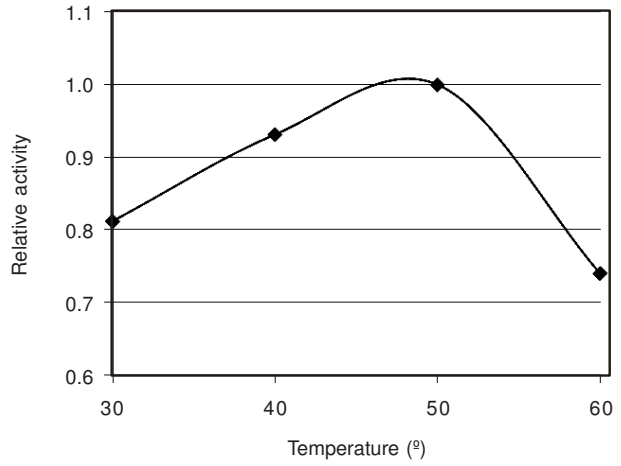

Fig. 2. Effect of temperature on soyabean peroxidase activity

Effect of pH on soybean hulls peroxidase activity: The stable pH for free soybean hulls peroxidase was screened by determining its activity in the pH ranges 4-8. The solution pH value was adjusted using 1 mol/L HCl/NaOH solution and the enzyme activity was evaluated from pH 3 to 7. Fig. 3 showed that soybean hulls peroxidase is relatively stable at pH 4-5. The activity of the enzyme was lower below pH 4 and above pH 5. This is consistent with other research reports which results the soybean hulls peroxidase activity is higher in weak acid conditions.

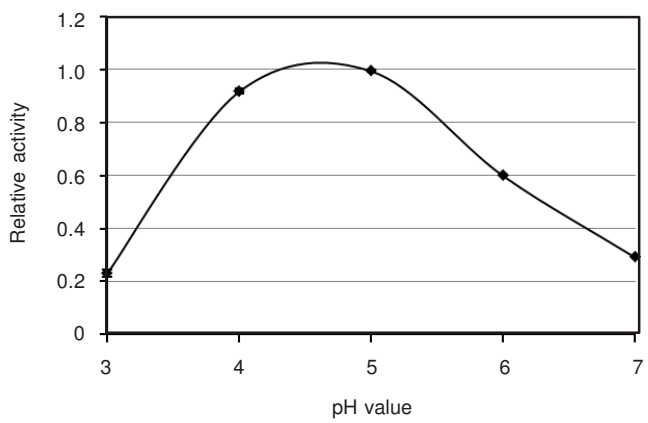

Fig. 3. Effect of pH value on soyabean peroxidase activity

Possible interactions between these experimental conditions were not considered. It will be determined in our future works.

Effect of the soybean hulls peroxidase concentration and treatment period on removal of bisphenol A: In this experiment, we investigate the removal of bisphenol A by soybean hulls peroxidase extraction and crude soybean hulls. At first, we only use the different amounts of soybean hulls were used to test the dependence of phenol removal on the soybean hulls peroxidase dosage (Fig. 4). Another important factor is the treatment time which gives a maximum removal percentage of bisphenol A. Meanwhile, 0.5, 1.0 and 2.0 g were chosen for the removal of 25 mL of 100 µg/mL bisphenol A solution. Obviously, the removal percentage of bisphenol A

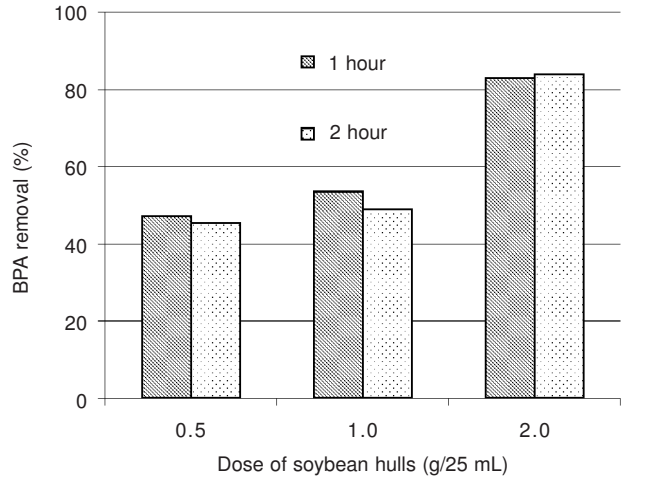

Fig. 4. Effect of the soyabean peroxidase concentration and treatment period on bisphenol A removal

by soybean hulls peroxidase in the presence of H_2O_2 is depending on the concentration of the enzyme amount, in this experiment the dosage is instead of enzyme amount.

Effect of initial H_2O_2 concentration on bisphenol A removal: In early researches[25,26], the optimum of molecular ratio of H_2O_2 to chemical compound phenol substrate is around 1 mmol/L. In this study, the optimization of H_2O_2 amount were detected in the absence of H_2O_2 and using initial concentrations of 0.5, 1, 5, 10 and 20 mmol/L at 2 h treatment period. In order to determine the effect of H_2O_2 on bisphenol A removal, we choose two different concentration of bisphenol A concentration (100 µg/mL and 200 µg/mL, respectively) removal experiment in the soybean hulls peroxidase concentration of 2 g/25 mL. However, in this study, we found more H_2O_2 was required to remove bisphenol A (Fig. 5). Since the crude extract contains a mixture of multiple soybean proteins, soybean seed hull slurry required a higher concentration of H_2O_2 to remove the organic substrates than did the purified enzyme. However, much more H_2O_2 had not helped to significantly improve bisphenol A removal in this experiment. It is presumed that H_2O_2 can inactive the soybean hulls peroxidase as a kind of strong oxidizer.

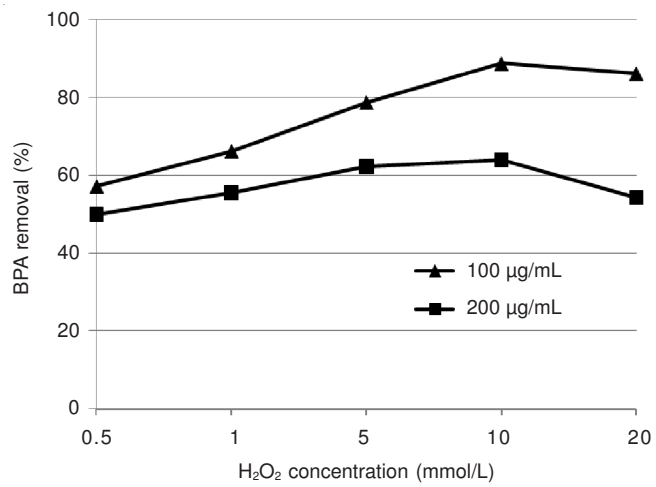

Fig. 5. Effect of initial H_2O_2 concentration on BPA removal

Conclusion

This study demonstrated that crude soybean hulls peroxidase and soybean hulls are an abundant and available source of peroxidase. Soybean hulls are by-products of food industry. It can be easily removed from soybean seeds and dissolve in water solution. The soybean hulls can be applied in the removal of chemical compounds directly.

Soybean hulls peroxidase has relative high activity at weak acid pH and high temperature experimental conditions. Based on these advantages, soybean hulls peroxidase has a potential to apply on treatment of high temperature and low pH value effluents directly.

Soybean hulls peroxidase was found to have positive effect on removal of bisphenol A from simulation wastewater at relative higher concentration conditions.

The performance of soybean hulls peroxidase on bisphenol A removal depends on the soybean hulls dosage and the initial concentration of H_2O_2.

In conclusion, crude soybean hulls peroxidase is an efficient, economical and environment friendly material of removal in aqueous bisphenol A treatment. This study is only focused on laboratory experiment, more studies of treatment for real effluent are needed in future experiment, especially reactor technologies.

ACKNOWLEDGEMENTS

The study was supported by Key Laboratory of Three Gorges Reservoir Region's Eco-Environment Ministry of Education. It has been also financially supported by China Scholarship Council in form of scholarship.

REFERENCES

1. J.C. O'Connor and R.E. Chapin, *Pure Appl. Chem.*, **75**, 2099 (2003).
2. I.D. Buchanan, J.A. Nicell and M. Wagner, *J. Environ. Eng.-ASCE*, **124**, 794 (1998).
3. J. Dec and J.M. Bollag, *Arch. Environ. Contaminat. Toxicol.*, **19**, 543 (1990).
4. A.M. Klibanov, T.M. Tu and K.P. Scott, *Science*, **221**, 259 (1983).
5. J.S. Dordick, M.A. Marletta and A.M. Klibanov, *Resour., Conservat. Recyc.*, **5**, 195 (1991).
6. J.A. Nicell, J.K. Bewtra, K.E. Taylor, N. Biswas and C. St.Pierre, *Water Sci. Technol.*, **25, No. 3**, 157 (1992).
7. S.W. Maloney, J. Manem, J. Mallevialle and F. Fiessinge, *Environ. Sci. Technol.*, **20**, 249 (1986).
8. A.M. Klibanov and E.D. Morris, *Enzyme Microb. Technol.*, **3**, 119 (1981).
9. A. Bódalo, J. Bastida, M.F. Máximo, M.C. Montiel, M. Gómez and M.D. Murcia, *Bioprocess Biosyst. Eng.*, **31**, 587 (2008).
10. D.J. Sessa and R.L. Anderson, *J. Agric. Food Chem.*, **29**, 960 (1981).
11. J.W. Gilikin and J.S. Graham, *Plant Physiol*., **96**, 214 (1991).
12. M. Gijzen, R. Van Huystee and R.I. Buzzell, *Plant Physiol*., **103**, 1061 (1993).
13. J.P. McEldoon and J.S. Dordick, *Biotechnol. Prog.*, **12**, 555 (1996).
14. N. Caza, J.K. Bewtra, N. Biswas and K.E. Taylor, *Water Res.*, **33**, 3012 (1999).
15. C. Flock, A. Bassi and M. Gijzen, *J. Chem. Technol. Biotechnol.*, **74**, 303 (1999).
16. H. Wright and J.A. Nicell, *Bioresour. Technol.*, **70**, 69 (1999).
17. A. Bassi, Z. Geng and M. Gijzen, *Eng. Life Sci.*, **4**, 125 (2004).
18. A. Bódalo, J.L. Gómez, E. Gómez, A.M. Hidalgo, M. Gómez and A.M. Yelo, *Desalination*, **195**, 51 (2006).
19. J.W. Gilikin and J.S. Graham, *Plant Physiol.*, **96**, 214 (1991).
20. J.E. Biles, T.P. McNeal, T.H. Begley and H.C. Hollifield, *J. Agric. Food Chem.*, **45**, 3541 (1997).
21. J. Sajiki, K. Takahashi and J. Yonekubo, *J. Chromatogr. B*, **736**, 255 (1999).
22. V.A. Cooper and J.A. Nicell, *Water Res.*, **30**, 954 (1996).
23. E. Miland, M.R. Smyth and C.O. Fagain, *J. Chem. Technol. Biotechnol.*, **67**, 227 (1996).
24. J.P. McEldoon, A.R. Pokora and J.S. Dordick, *Enzyme Microb. Technol.*, **17**, 359 (1995).
25. J.A. Nicell, *et al.*, *Biodeterioration Abstr.*, **7**, 1 (1993).
26. I.D. Buchanan and J.A. Nicell, *Biotechnol. Bioeng.*, **54**, 251 (1997).